

Overview: The DNA Toolbox

- Sequencing of the human genome was completed by 2007
- DNA sequencing has depended on advances in technology, starting with making recombinant DNA
- In **recombinant DNA**, nucleotide sequences from two different sources, often two species, are combined *in vitro* into the same DNA molecule

Concept 20.1: DNA cloning yields multiple copies of a gene or other DNA segment

- To work directly with specific genes, scientists prepare gene-sized pieces of DNA in identical copies, a process called *DNA cloning*

DNA Cloning and Its Applications: *A Preview*

- Most methods for cloning pieces of DNA in the laboratory share general features, such as the use of bacteria and their plasmids
- **Plasmids** are small circular DNA molecules that replicate separately from the bacterial chromosome
- Cloned genes are useful for making copies of a particular gene and producing a protein product

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- **Gene cloning** involves using bacteria to make multiple copies of a gene
 - Foreign DNA is inserted into a plasmid, and the recombinant plasmid is inserted into a bacterial cell
 - Reproduction in the bacterial cell results in cloning of the plasmid including the foreign DNA
 - This results in the production of multiple copies of a single gene

Fig. 20-2a

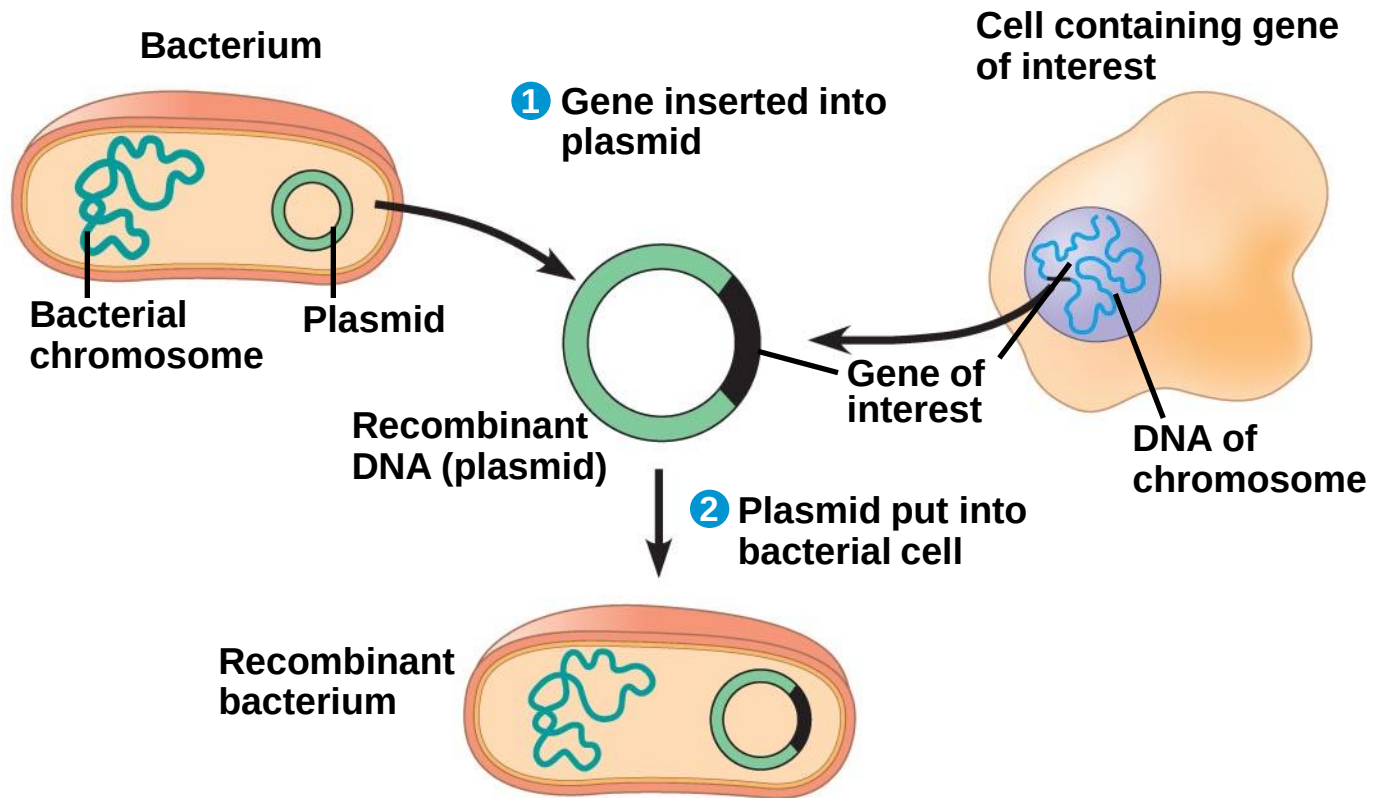
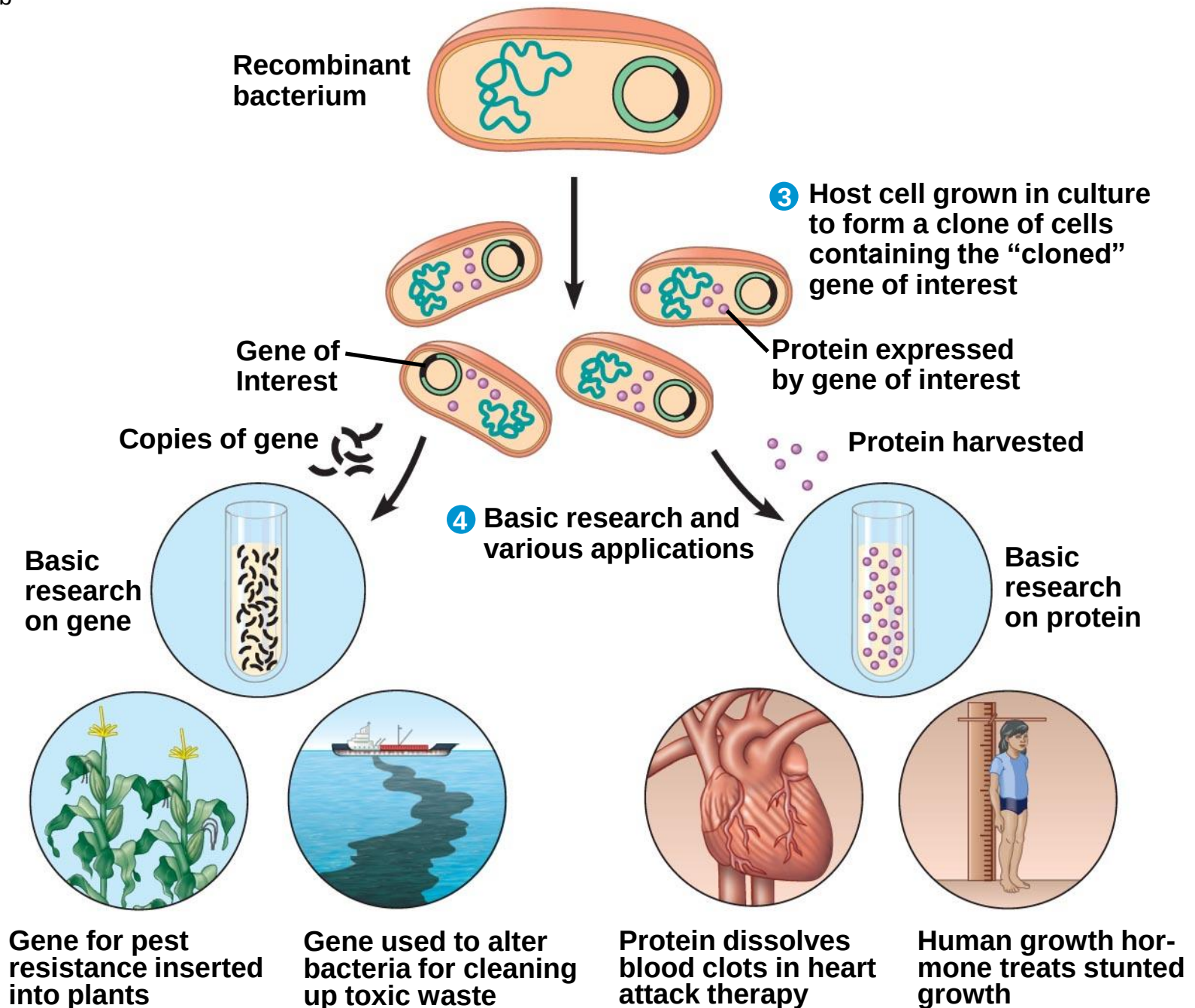


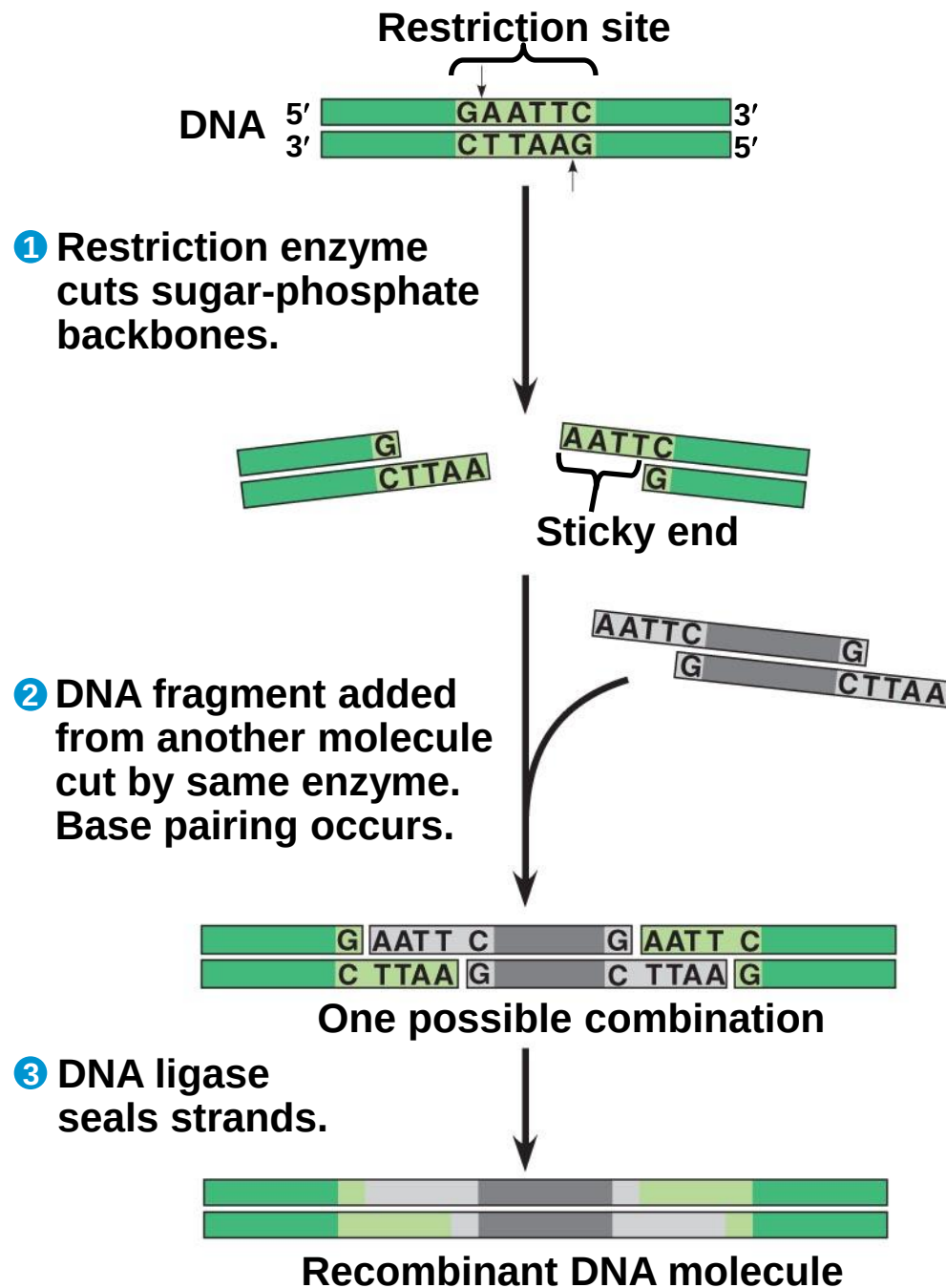
Fig. 20-2b



Using Restriction Enzymes to Make Recombinant DNA

- Bacterial **restriction enzymes** cut DNA molecules at specific DNA sequences called **restriction sites**
 - Bacteria protect their own DNA by methylating the sequences recognized by these enzymes.
- A restriction enzyme usually makes many cuts, yielding **restriction fragments**

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- The most useful restriction enzymes cut DNA in a staggered way, producing fragments with “**sticky ends**” that bond with complementary sticky ends of other fragments
 - **DNA ligase** is an enzyme that seals the bonds between restriction fragments



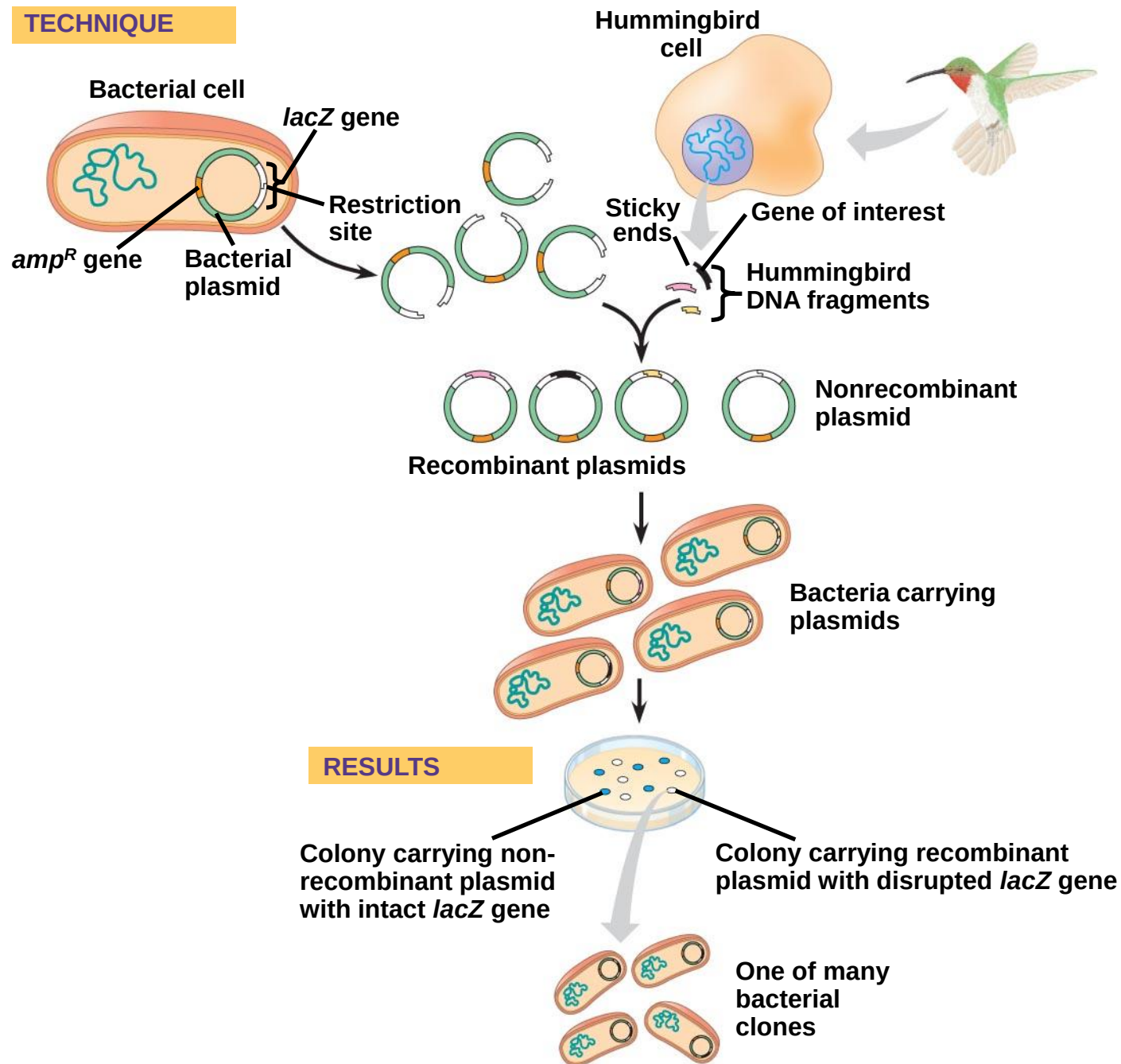
Cloning a Eukaryotic Gene in a Bacterial Plasmid

- In gene cloning, the original plasmid is called a **cloning vector**
- A cloning vector is a DNA molecule that can carry foreign DNA into a host cell and replicate there

Producing Clones of Cells Carrying Recombinant Plasmids

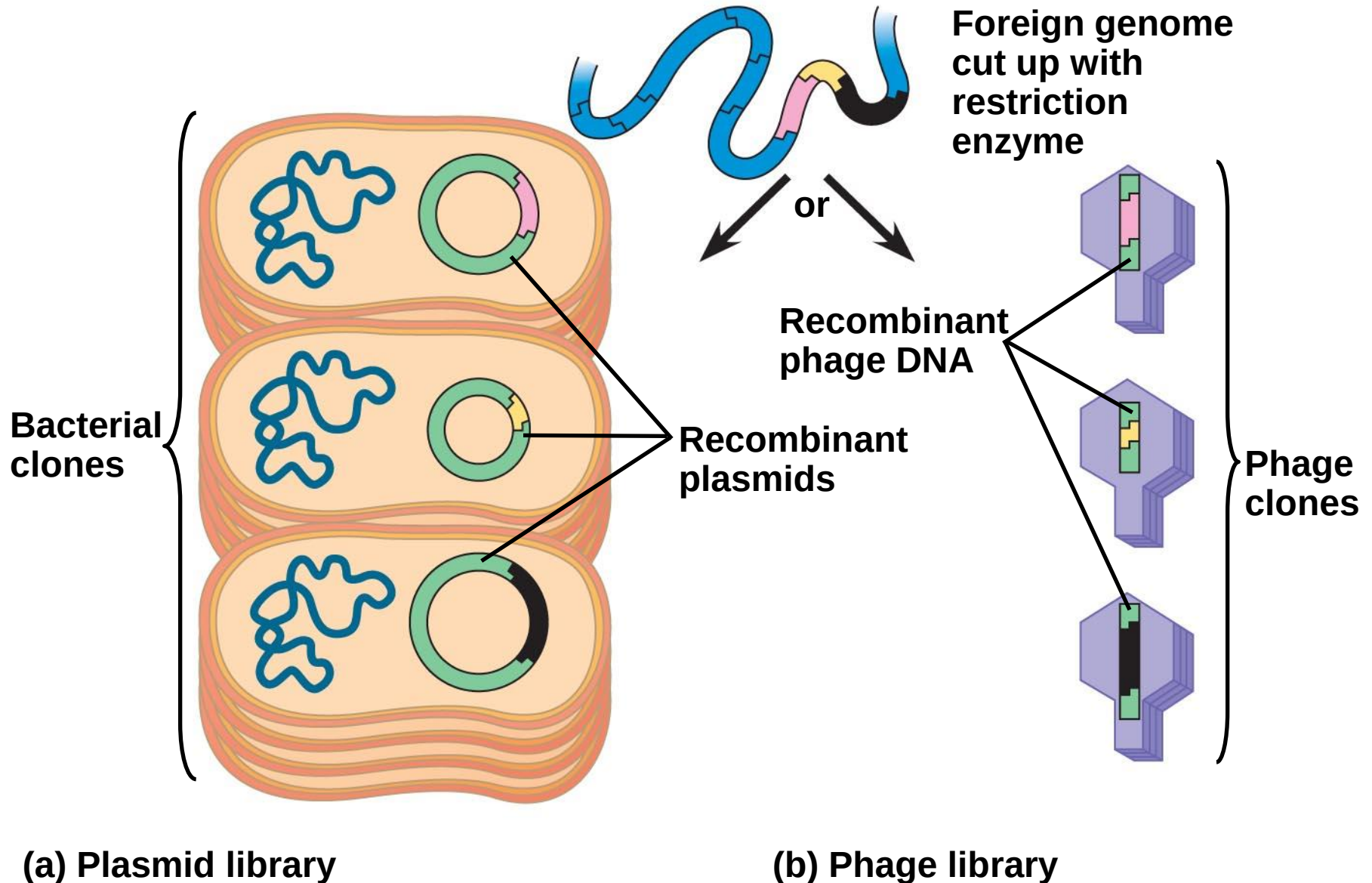
- Several steps are required to clone the β -globin gene in a bacterial plasmid:
 - The genomic DNA and a bacterial plasmid are isolated
 - Both are digested with the same restriction enzyme
 - The fragments are mixed, and DNA ligase is added to bond the fragment sticky ends

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- Some recombinant plasmids now contain this type of DNA
 - The DNA mixture is added to bacteria that have been genetically engineered to accept it
 - The bacteria are plated on a type of agar that selects for the bacteria with recombinant plasmids
 - This results in the cloning of many DNA fragments, including the β -globin gene

TECHNIQUE

Storing Cloned Genes in DNA Libraries

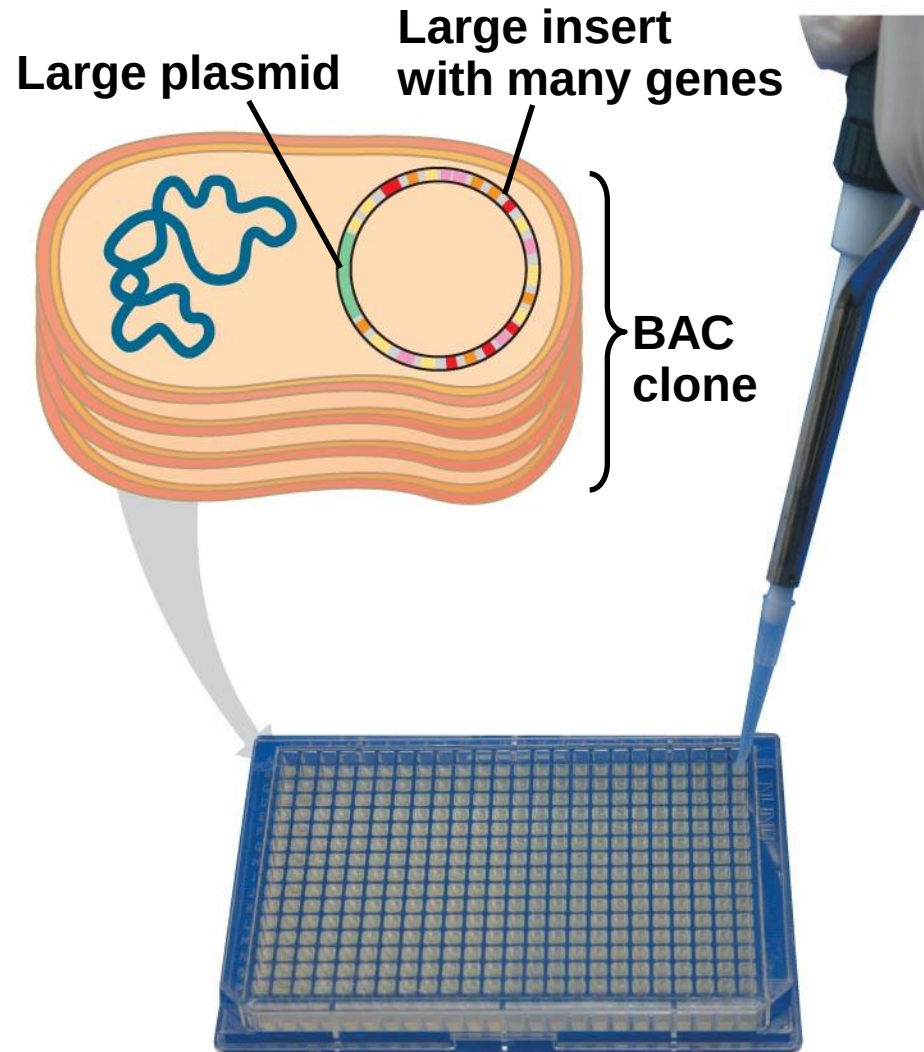
- A **genomic library** that is made using bacteria is the collection of recombinant vector clones produced by cloning DNA fragments from an entire genome
- A genomic library that is made using bacteriophages is stored as a collection of phage clones



(a) Plasmid library

(b) Phage library

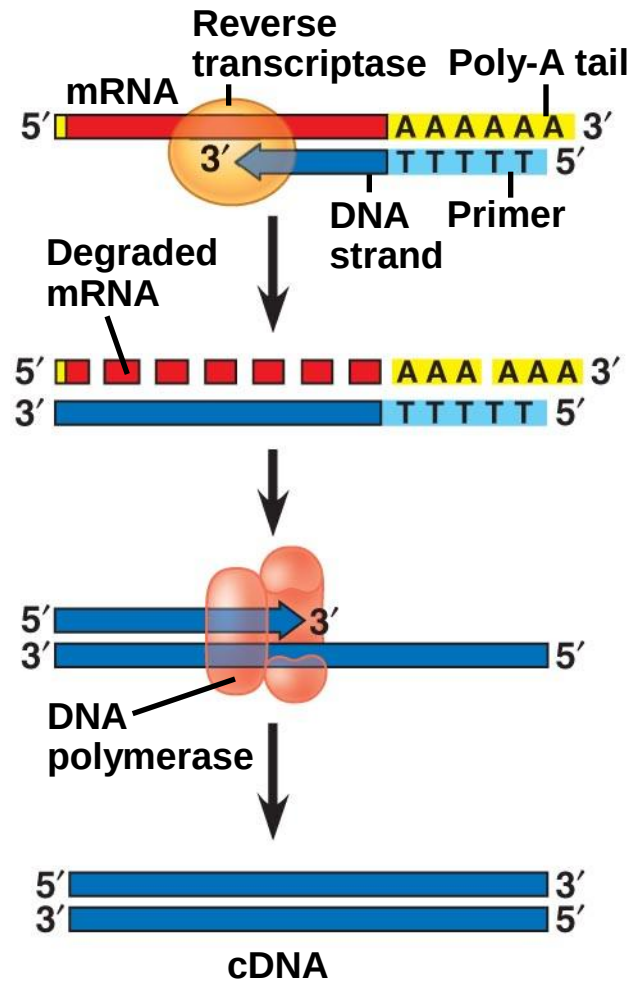
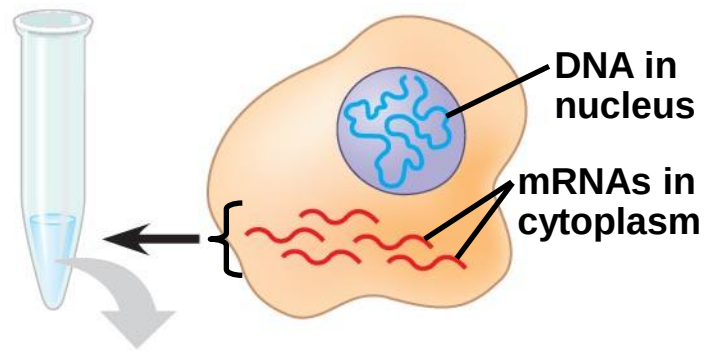
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- A **bacterial artificial chromosome (BAC)** is a large plasmid that has been trimmed down and can carry a large DNA insert
 - BACs are another type of vector used in DNA library construction



(c) A library of bacterial artificial chromosome (BAC) clones

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- A **complementary DNA (cDNA)** library is made by cloning DNA made *in vitro* by reverse transcription of all the mRNA produced by a particular cell
 - A **cDNA library** represents only part of the genome—only the subset of genes transcribed into mRNA in the original cells

Fig. 20-6-5



Genomic vs. cDNA libraries

If a researcher wants to clone a gene but is unsure in what cell type it is expressed or unable to obtain that cell type, a genomic library will likely contain the gene.

A researcher interested in the regulatory sequences or introns associated with a gene needs to obtain the gene from a genomic library.

- These sequences are missing from the processed mRNAs used in making a cDNA library.

A researcher interested in only the coding sequence of a gene can obtain a stripped-down version of the gene from a cDNA library.

Screening a Library for Clones Carrying a Gene of Interest

- A clone carrying the gene of interest can be identified with a **nucleic acid probe** having a sequence complementary to the gene
- This process is called **nucleic acid hybridization**
- A radioactive or fluorescent tag is used to label the probe

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- A probe can be synthesized that is complementary to the gene of interest
 - For example, if the desired gene is

5' ...GGCTAACTTAGC... 3'

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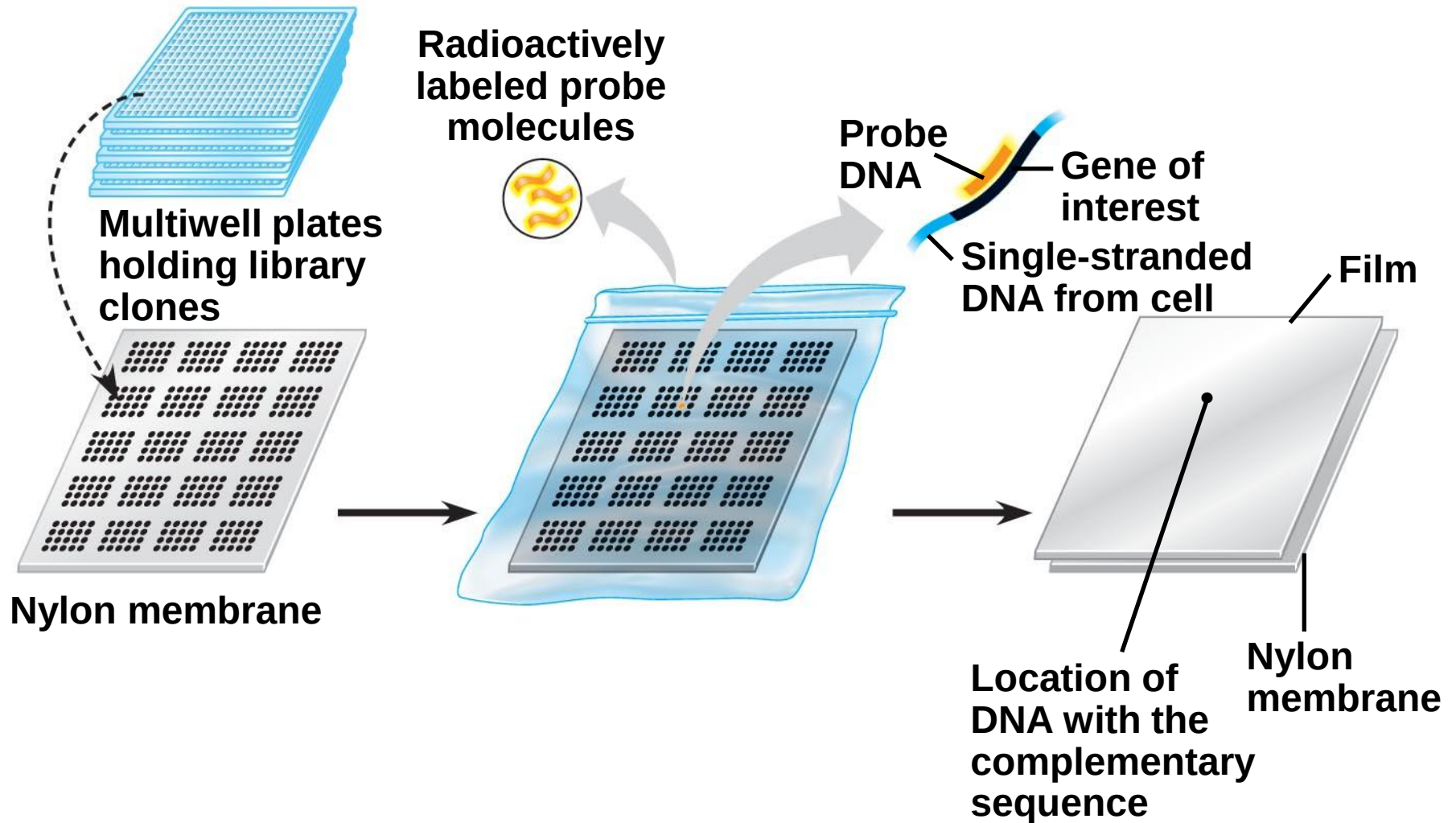
- Then we would synthesize this tagged probe

3' CCGATTGAATCG 5'

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- The DNA probe can be used to screen a large number of clones simultaneously for the gene of interest
 - Once identified, the clone carrying the gene of interest can be cultured

TECHNIQUE



Expressing Cloned Eukaryotic Genes

- After a gene has been cloned, its protein product can be produced in larger amounts for research
- Cloned genes can be expressed as protein in either bacterial or eukaryotic cells

Bacterial Expression Systems

- Several technical difficulties hinder expression of cloned eukaryotic genes in bacterial host cells
- To overcome differences in promoters and other DNA control sequences, scientists usually employ an **expression vector**, a cloning vector that contains a highly active prokaryotic promoter

Eukaryotic Cloning and Expression Systems

- The use of cultured eukaryotic cells as host cells and **yeast artificial chromosomes (YACs)** as vectors helps avoid gene expression problems
- YACs behave normally in mitosis and can carry more DNA than a plasmid
- Eukaryotic hosts can provide the post-translational modifications that many proteins require

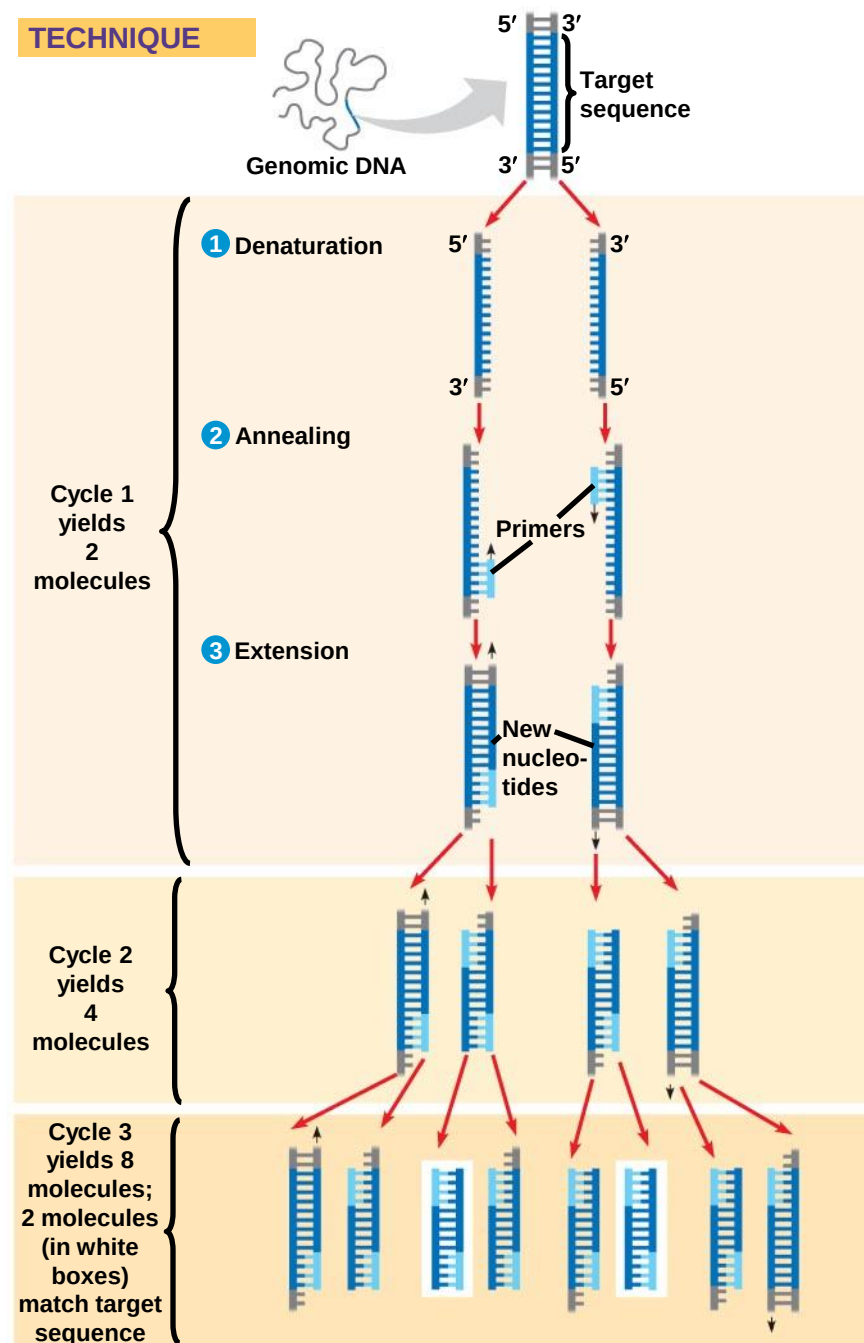
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- One method of introducing recombinant DNA into eukaryotic cells is **electroporation**, applying a brief electrical pulse to create temporary holes in plasma membranes
 - Alternatively, scientists can inject DNA into cells using microscopically thin needles
 - Once inside the cell, the DNA is incorporated into the cell's DNA by natural genetic recombination

Amplifying DNA *in Vitro*: The Polymerase Chain Reaction (PCR)

- The **polymerase chain reaction, PCR**, can produce many copies of a specific target segment of DNA
- A three-step cycle—heating, cooling, and replication—brings about a chain reaction that produces an exponentially growing population of identical DNA molecules

Fig. 20-8

TECHNIQUE



Concept 20.2: DNA technology allows us to study the sequence, expression, and function of a gene

- DNA cloning allows researchers to
 - Compare genes and alleles between individuals
 - Locate gene expression in a body
 - Determine the role of a gene in an organism
- Several techniques are used to analyze the DNA of genes

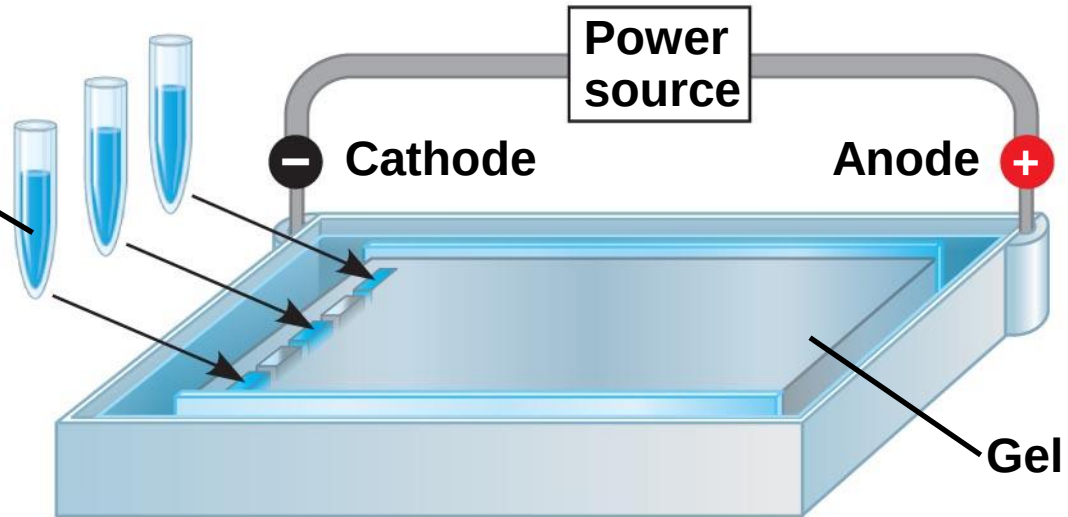
Gel Electrophoresis and Southern Blotting

- One indirect method of rapidly analyzing and comparing genomes is **gel electrophoresis**
- This technique uses a gel as a molecular sieve to separate nucleic acids or proteins by size
- A current is applied that causes charged molecules to move through the gel
- Molecules are sorted into “bands” by their size

TECHNIQUE

Mixture of
DNA mol-
ecules of
different
sizes

1



Longer
molecules

2

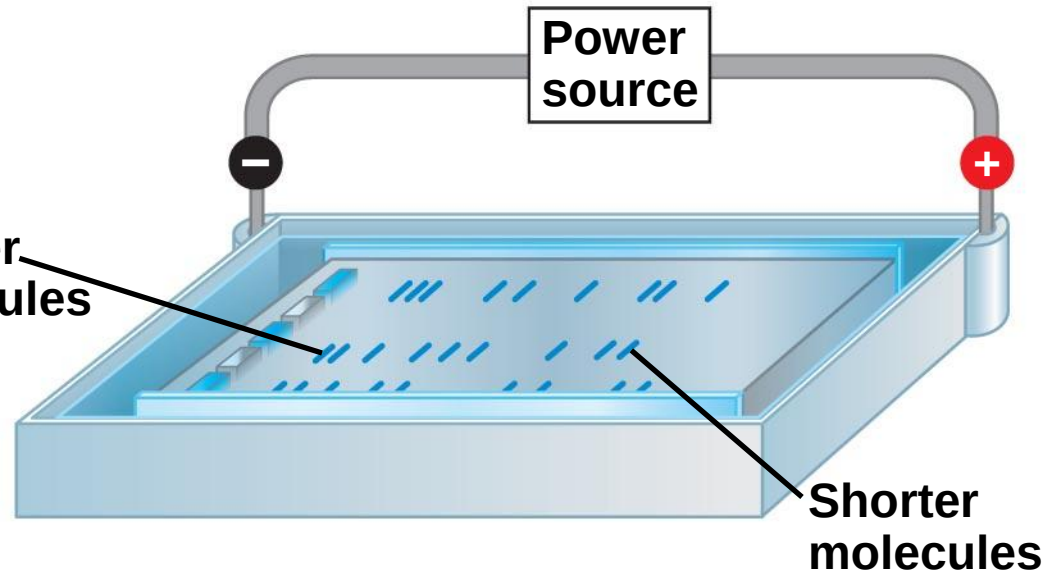
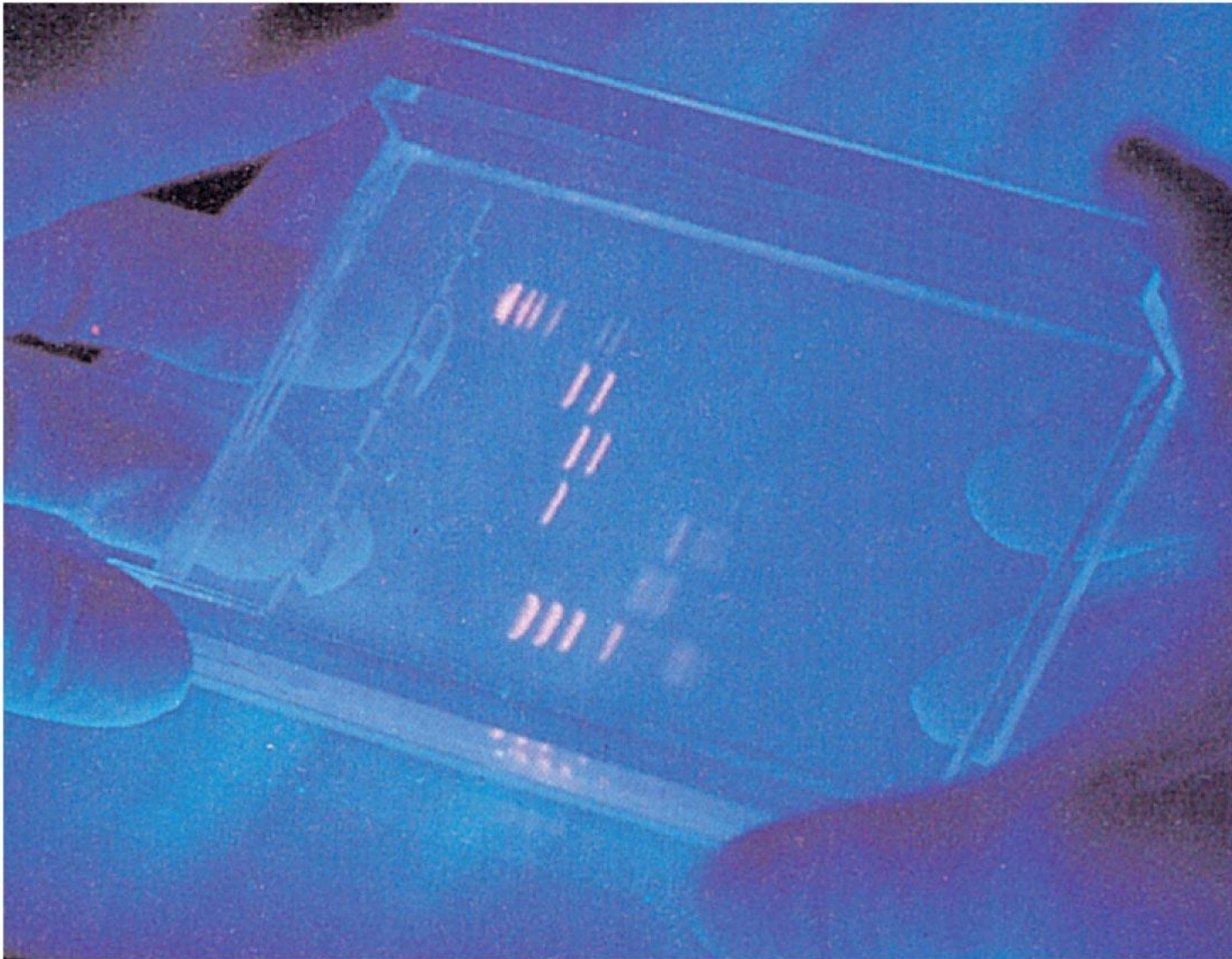


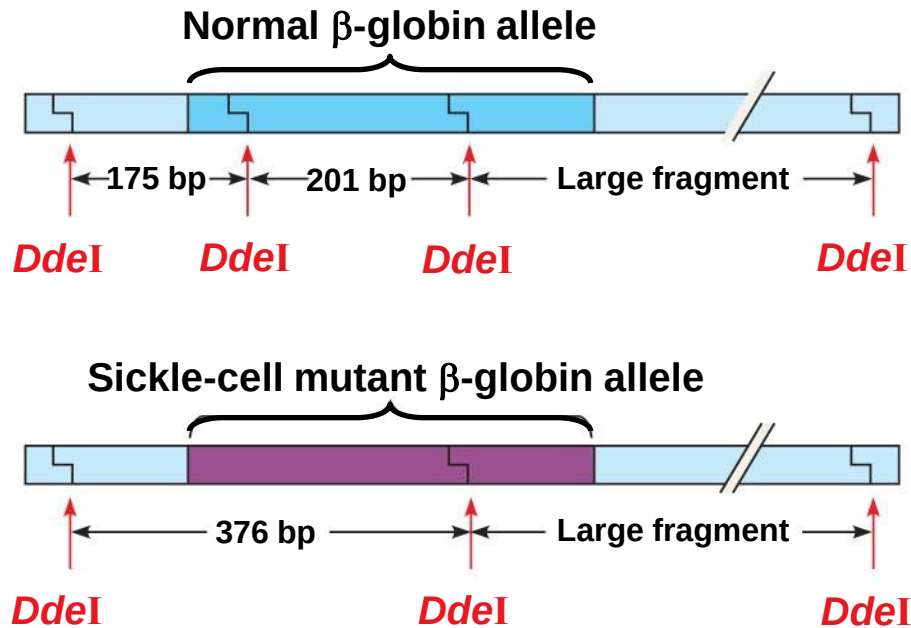
Fig. 20-9b

RESULTS

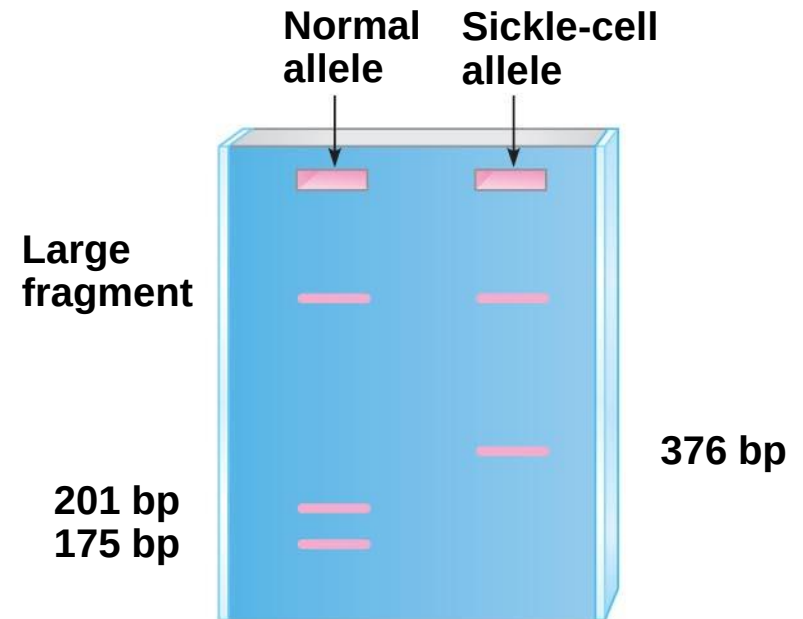


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- In *restriction fragment analysis*, DNA fragments produced by restriction enzyme digestion of a DNA molecule are sorted by gel electrophoresis
 - Restriction fragment analysis is useful for comparing two different DNA molecules, such as two alleles for a gene
 - The procedure is also used to prepare pure samples of individual fragments



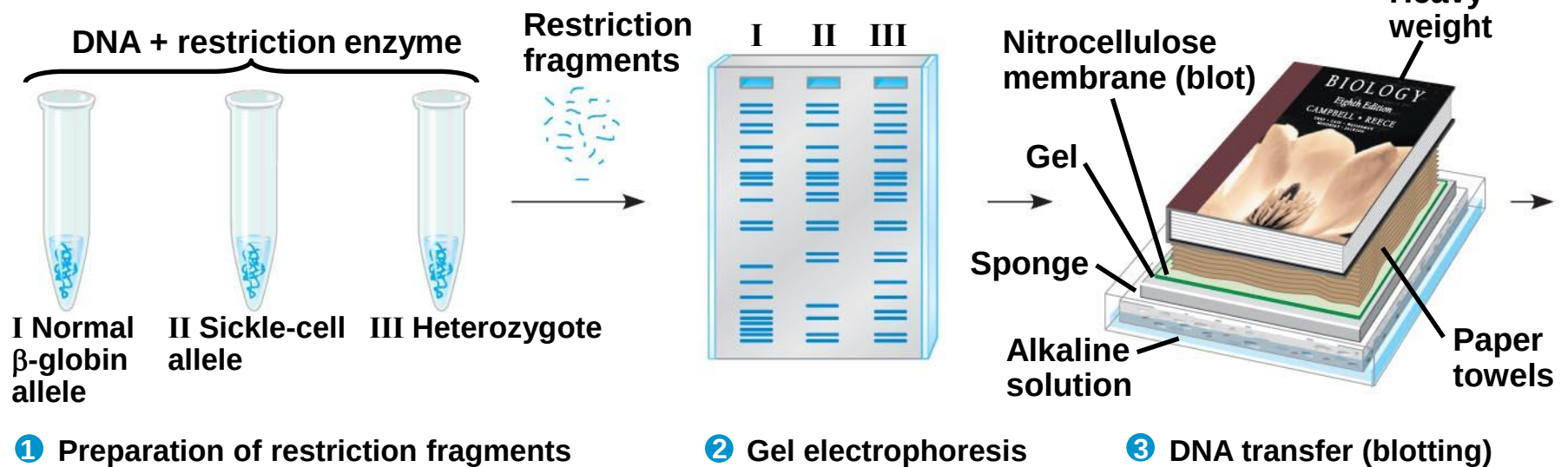
(a) *DdeI* restriction sites in normal and sickle-cell alleles of β -globin gene

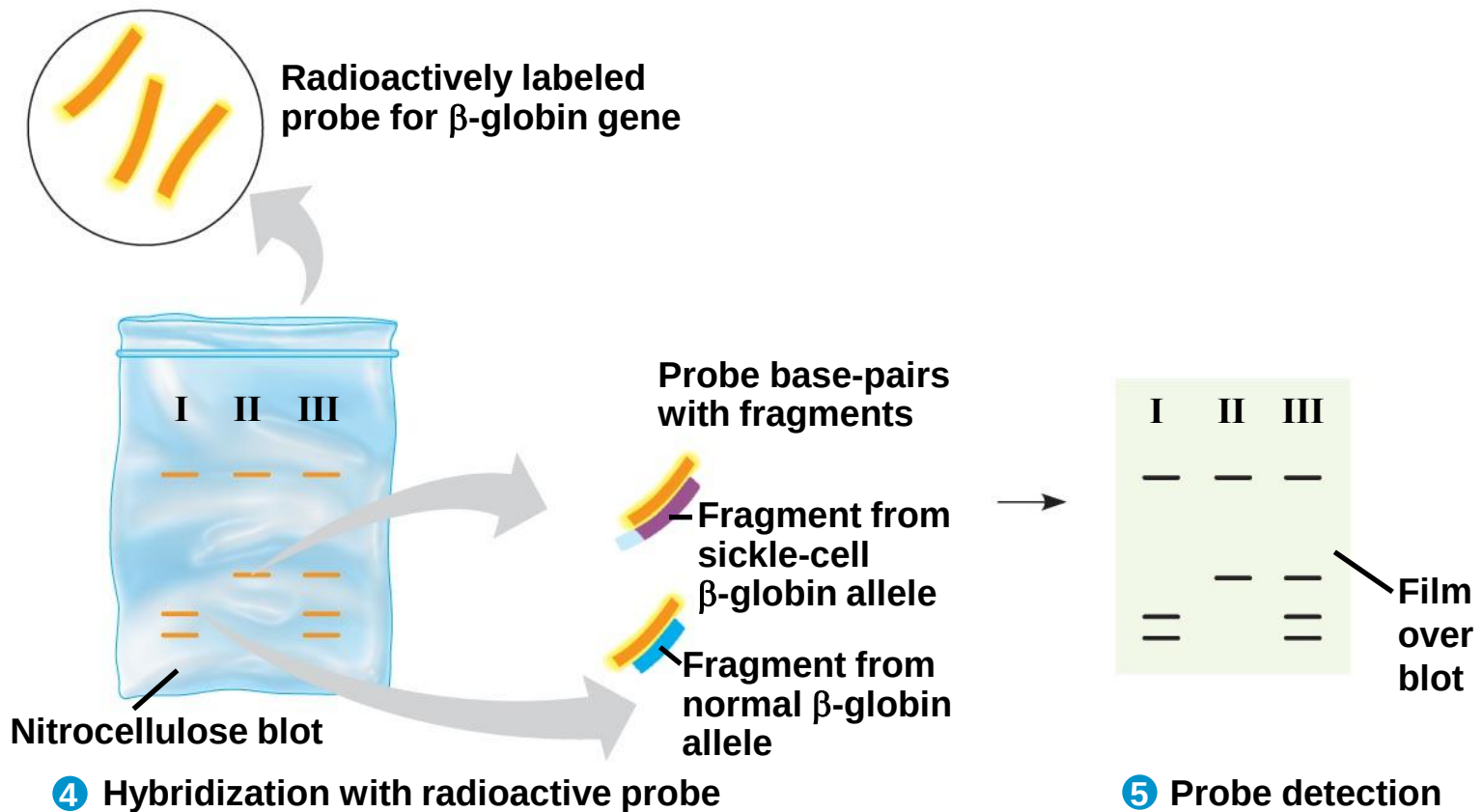


(b) Electrophoresis of restriction fragments from normal and sickle-cell alleles

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- A technique called **Southern blotting** combines gel electrophoresis of DNA fragments with nucleic acid hybridization
 - Specific DNA fragments can be identified by Southern blotting, using labeled probes that hybridize to the DNA immobilized on a “blot” of gel

TECHNIQUE



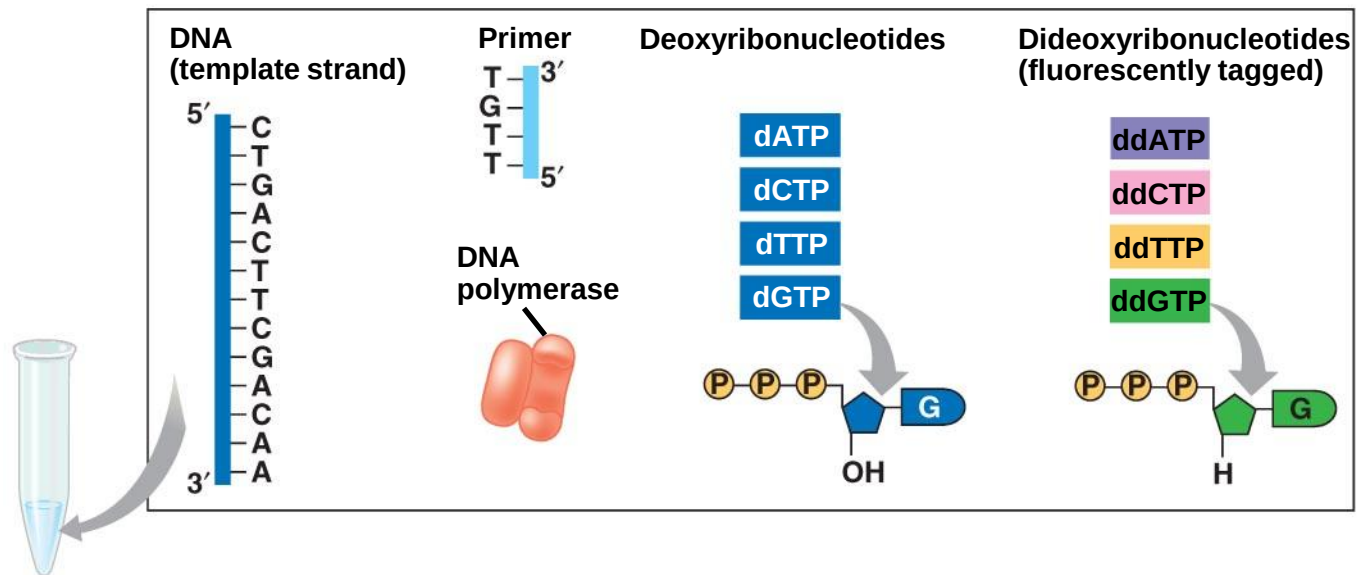


DNA Sequencing

- Relatively short DNA fragments can be sequenced by the *dideoxy chain termination method*
- Modified nucleotides called dideoxyribonucleotides (ddNTP) attach to synthesized DNA strands of different lengths
- Each type of ddNTP is tagged with a distinct fluorescent label that identifies the nucleotide at the end of each DNA fragment
- The DNA sequence can be read from the resulting spectrogram

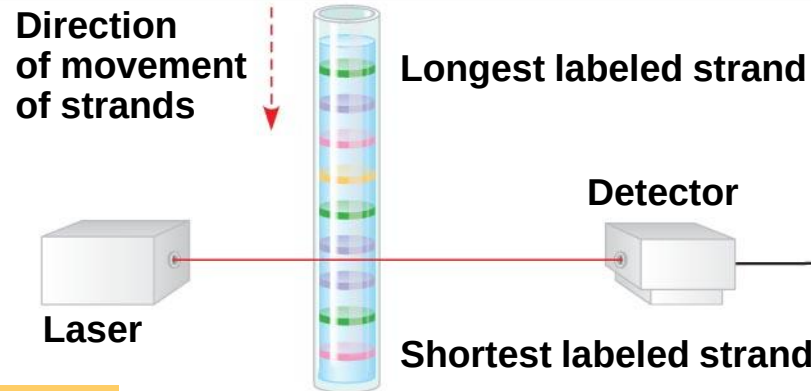
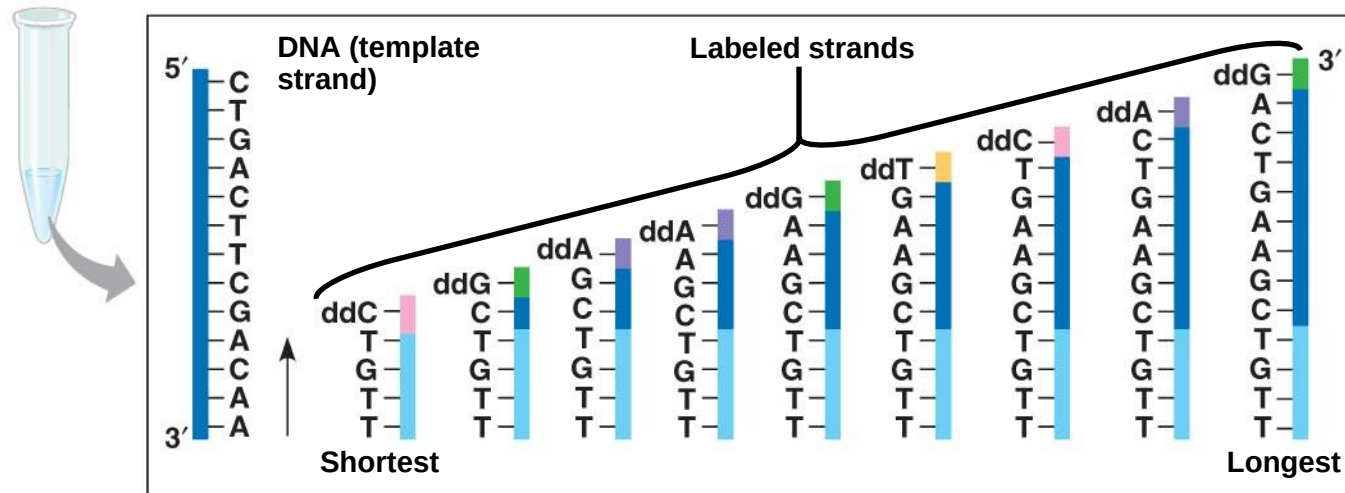
Fig. 20-12a

TECHNIQUE

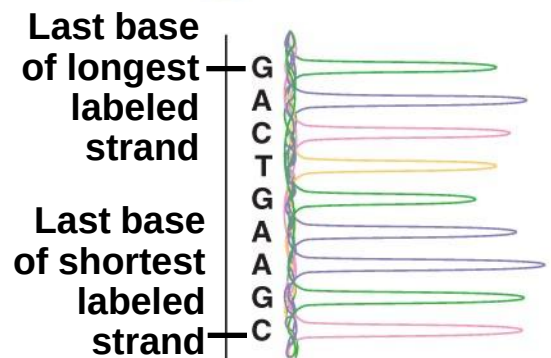


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TECHNIQUE



RESULTS



Analyzing Gene Expression

- Nucleic acid probes can hybridize with mRNAs transcribed from a gene
- Probes can be used to identify where or when a gene is transcribed in an organism

Studying the Expression of Single Genes

- Changes in the expression of a gene during embryonic development can be tested using
 - Northern blotting
 - Reverse transcriptase-polymerase chain reaction
- Both methods are used to compare mRNA from different developmental stages

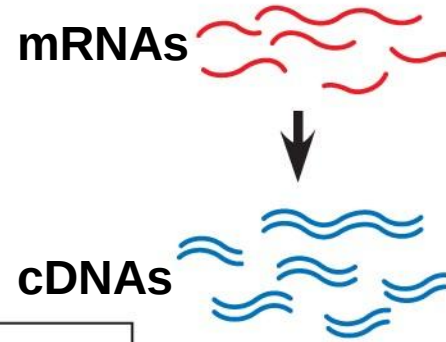
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- **Northern blotting** combines gel electrophoresis of mRNA followed by hybridization with a probe on a membrane
 - Identification of mRNA at a particular developmental stage suggests protein function at that stage

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- **Reverse transcriptase-polymerase chain reaction (RT-PCR)** is quicker and more sensitive
 - Reverse transcriptase is added to mRNA to make cDNA, which serves as a template for PCR amplification of the gene of interest
 - The products are run on a gel and the mRNA of interest identified

TECHNIQUE

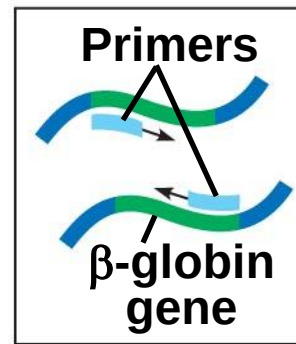
① cDNA synthesis

mRNAs



cDNAs

② PCR amplification

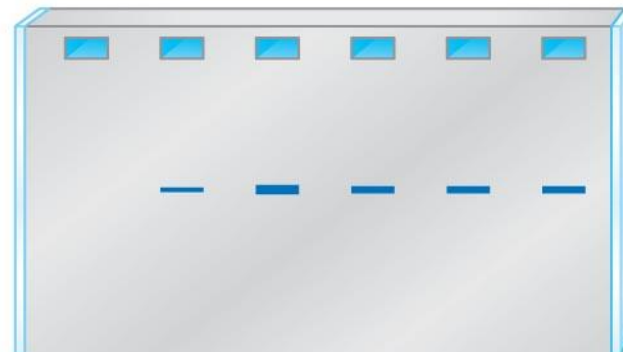


③ Gel electrophoresis

RESULTS

Embryonic stages

1 2 3 4 5 6



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- ***In situ* hybridization** uses fluorescent dyes attached to probes to identify the location of specific mRNAs in place in the intact organism

DNA Microarrays: Visualizing Gene Expression

- A DNA microarray allows visualization of gene expression.
- The pattern of glowing spots enables the researcher to determine which genes were being transcribed in the starting cells.
- Researchers can thus learn which genes are active in different tissues or in tissues from individuals in different states of health.

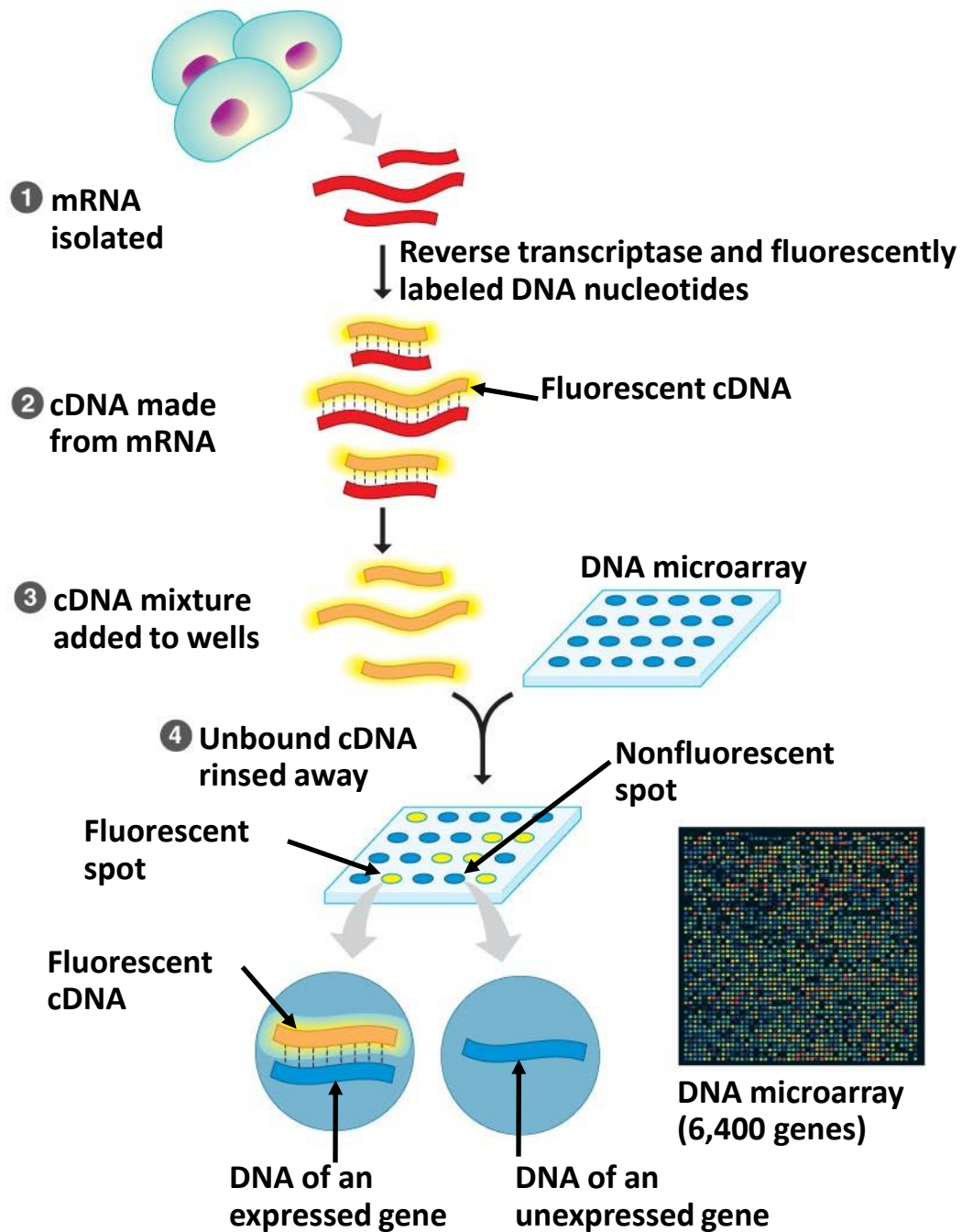
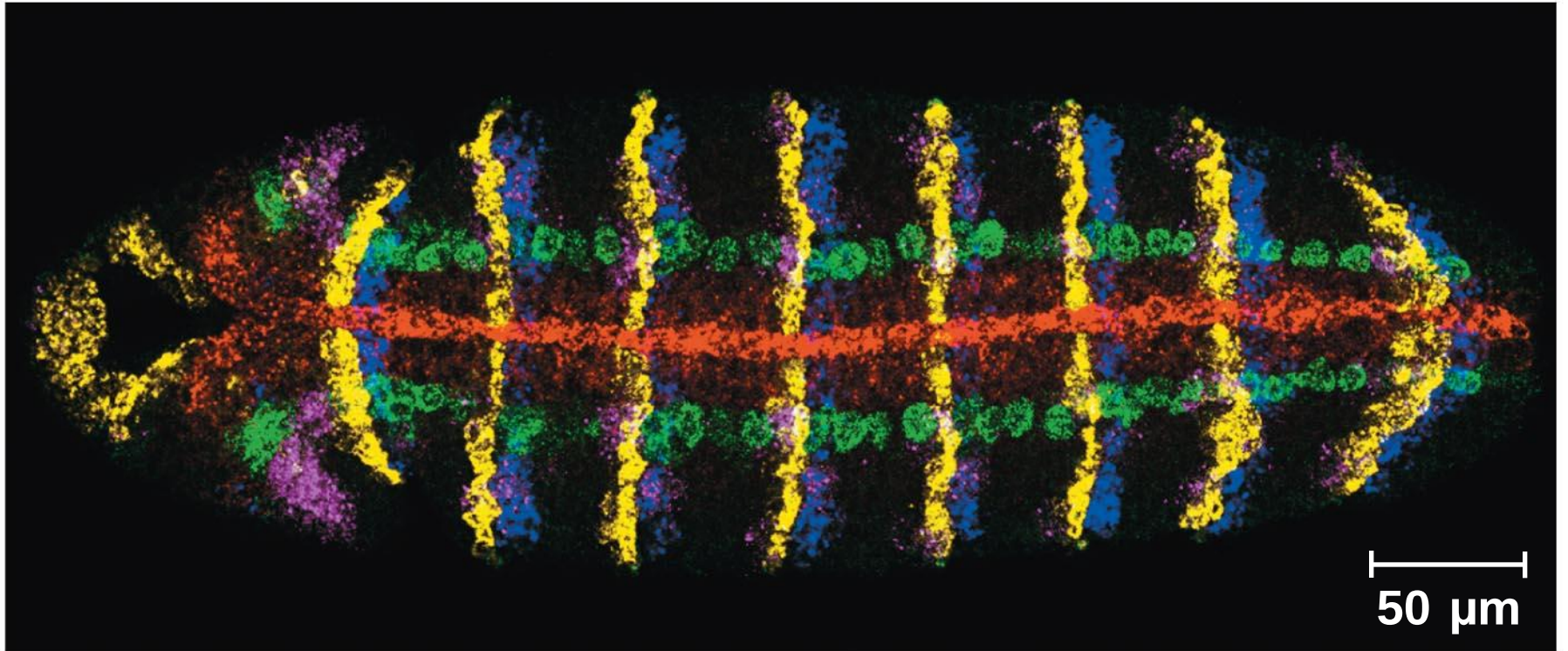


Fig. 20-14



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Studying the Expression of Interacting Groups of Genes

- Automation has allowed scientists to measure expression of thousands of genes at one time using DNA microarray assays
- **DNA microarray assays** compare patterns of gene expression in different tissues, at different times, or under different conditions

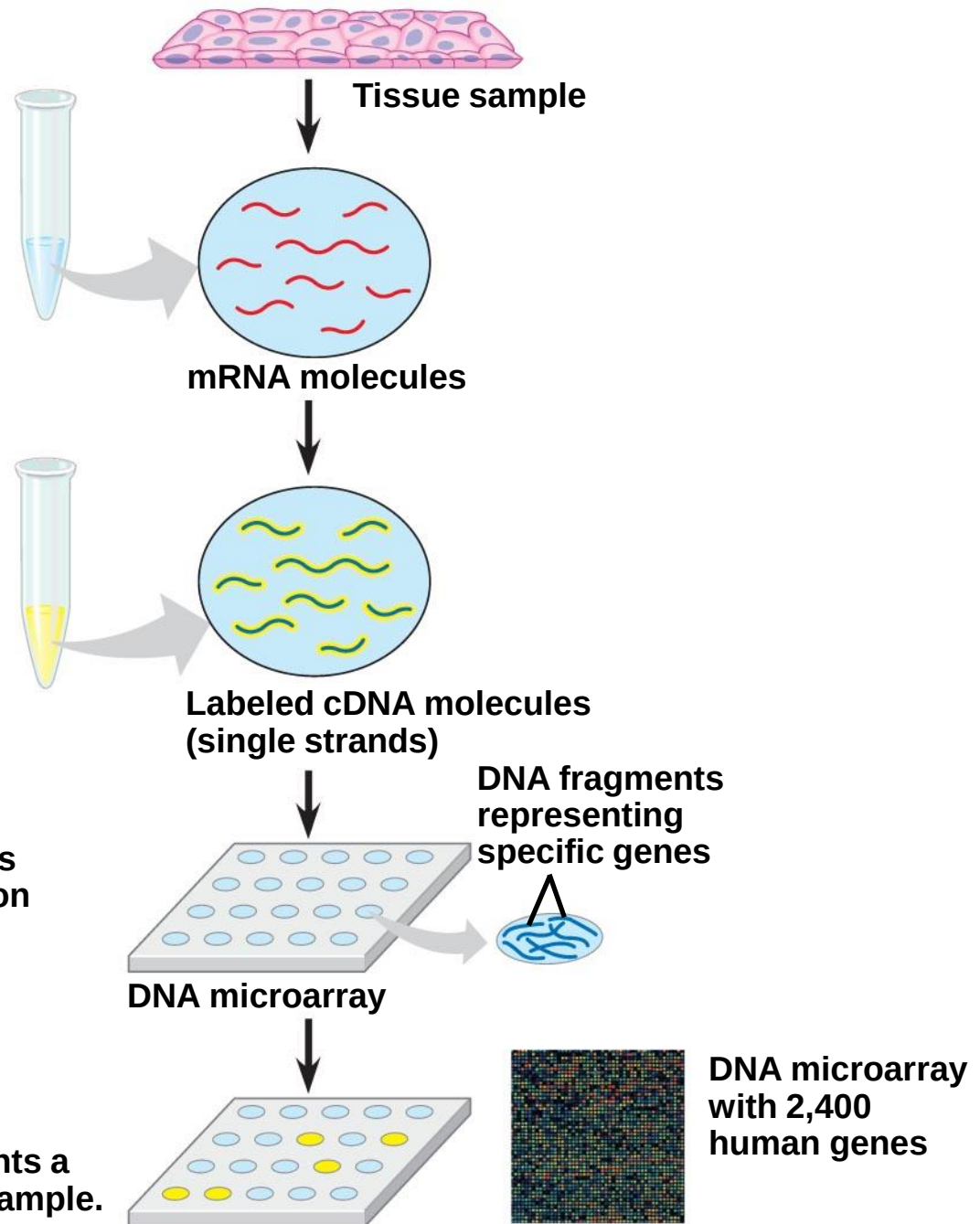
TECHNIQUE

1 Isolate mRNA.

2 Make cDNA by reverse transcription, using fluorescently labeled nucleotides.

3 Apply the cDNA mixture to a microarray, a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray.

4 Rinse off excess cDNA; scan microarray for fluorescence. Each fluorescent spot represents a gene expressed in the tissue sample.



Determining Gene Function

- One way to determine function is to disable the gene and observe the consequences
- Using ***in vitro* mutagenesis**, mutations are introduced into a cloned gene, altering or destroying its function
- When the mutated gene is returned to the cell, the normal gene's function might be determined by examining the mutant's phenotype

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- Gene expression can also be silenced using **RNA interference (RNAi)**
 - Synthetic double-stranded RNA molecules matching the sequence of a particular gene are used to break down or block the gene's mRNA

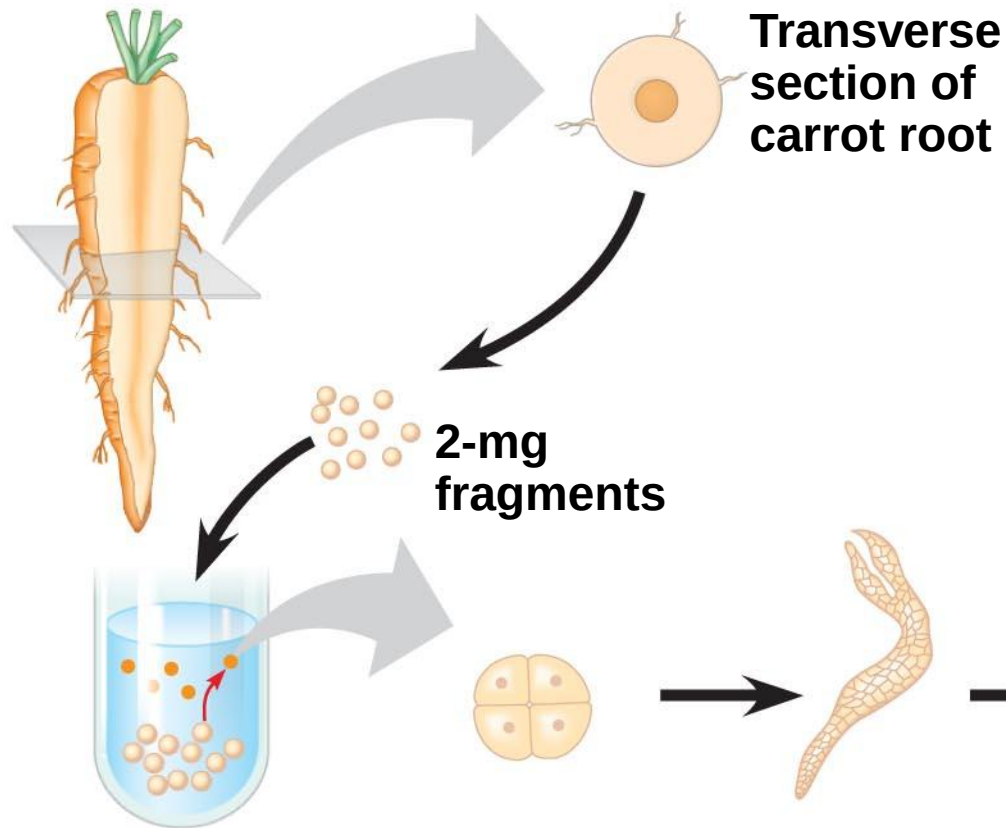
Concept 20.3: Cloning organisms may lead to production of stem cells for research and other applications

- Organismal cloning produces one or more organisms genetically identical to the “parent” that donated the single cell
 - Different from gene cloning (discussed previously) and cell cloning, (the division of an asexually reproducing cell into a collection of genetically identical cells).

Cloning Plants: Single-Cell Cultures

- One experimental approach for testing genomic equivalence is to see whether a differentiated cell can generate a whole organism
- A **totipotent** cell is one that can generate a complete new organism

EXPERIMENT



Fragments were cultured in nutrient medium; stirring caused single cells to shear off into the liquid.

Single cells free in suspension began to divide.

Embryonic plant developed from a cultured single cell.

Plantlet was cultured on agar medium. Later it was planted in soil.

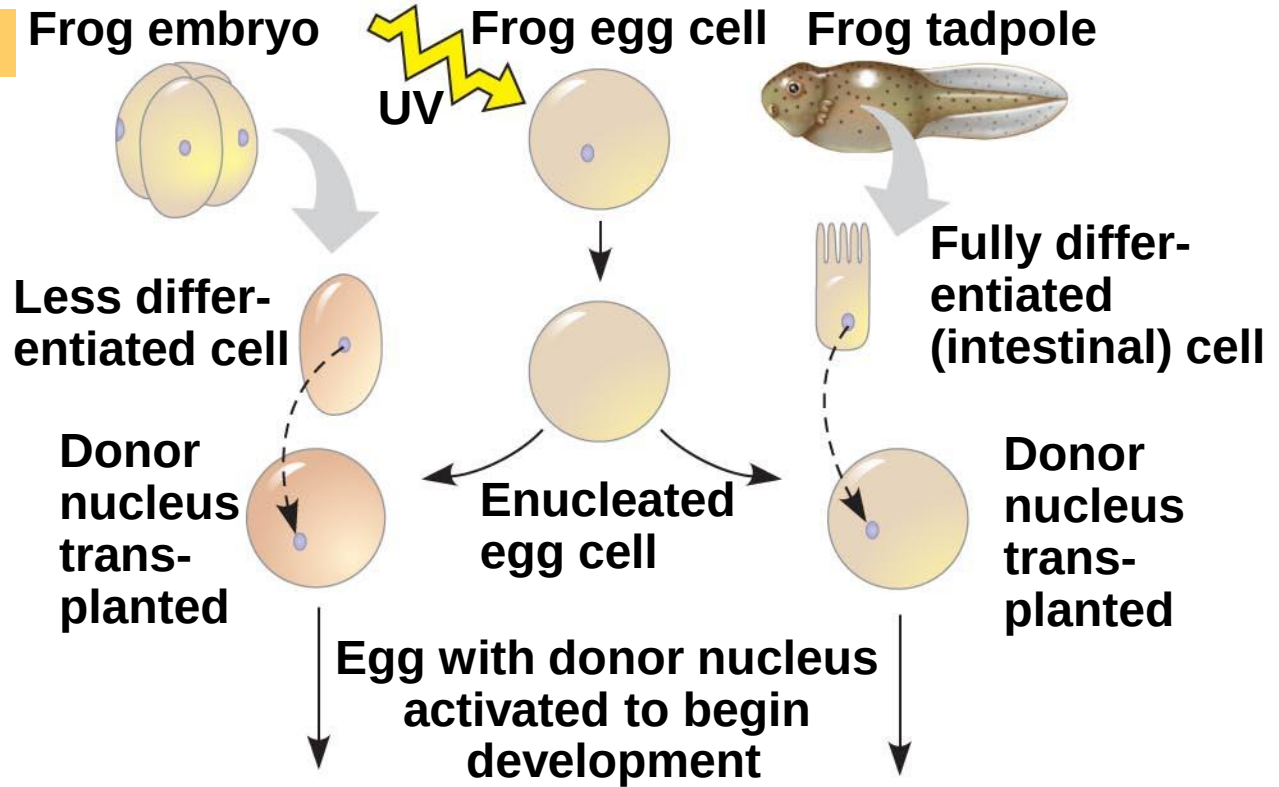
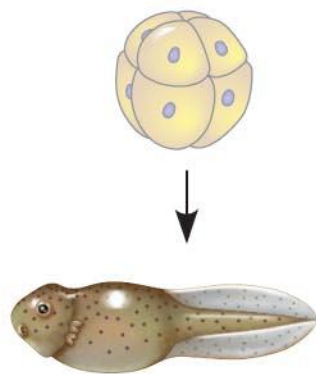
A single somatic carrot cell developed into a mature carrot plant.

RESULTS

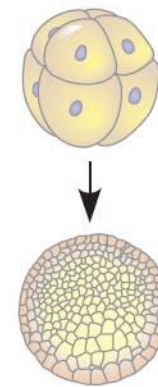


Cloning Animals: Nuclear Transplantation

- In nuclear transplantation, the nucleus of an unfertilized egg cell or zygote is replaced with the nucleus of a differentiated cell
- Experiments with frog embryos have shown that a transplanted nucleus can often support normal development of the egg
- However, the older the donor nucleus, the lower the percentage of normally developing tadpoles

EXPERIMENT**RESULTS**

**Most develop
into tadpoles**

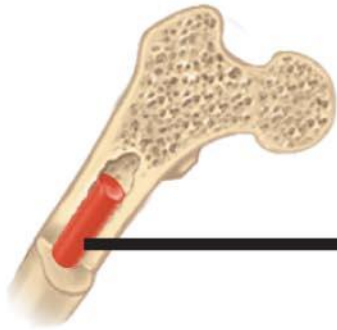


**Most stop developing
before tadpole stage**

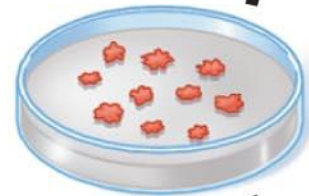
Reproductive Cloning of Mammals

- In 1997, Scottish researchers announced the birth of Dolly, a lamb cloned from an adult sheep by nuclear transplantation from a differentiated mammary cell
- Dolly's premature death in 2003, as well as her arthritis, led to speculation that her cells were not as healthy as those of a normal sheep, possibly reflecting incomplete reprogramming of the original transplanted nucleus

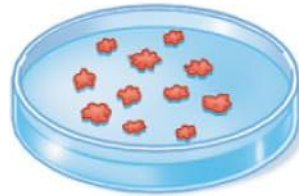
**Adult stem
cells in
bone marrow**



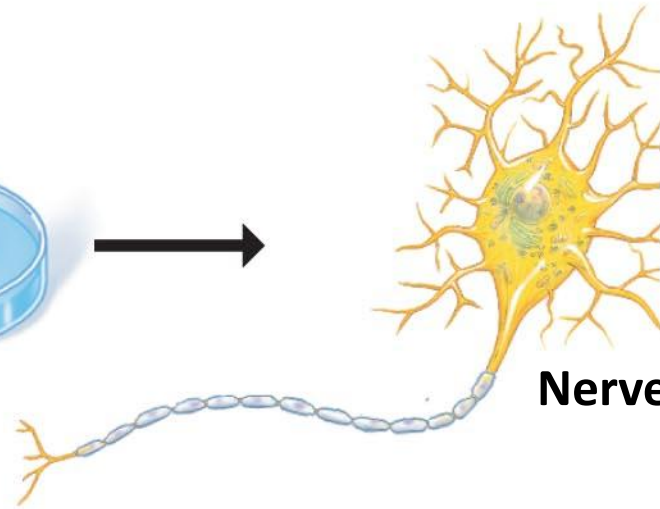
Blood cells



**Cultured
embryonic
stem cells**



Nerve cells



Heart muscle cells

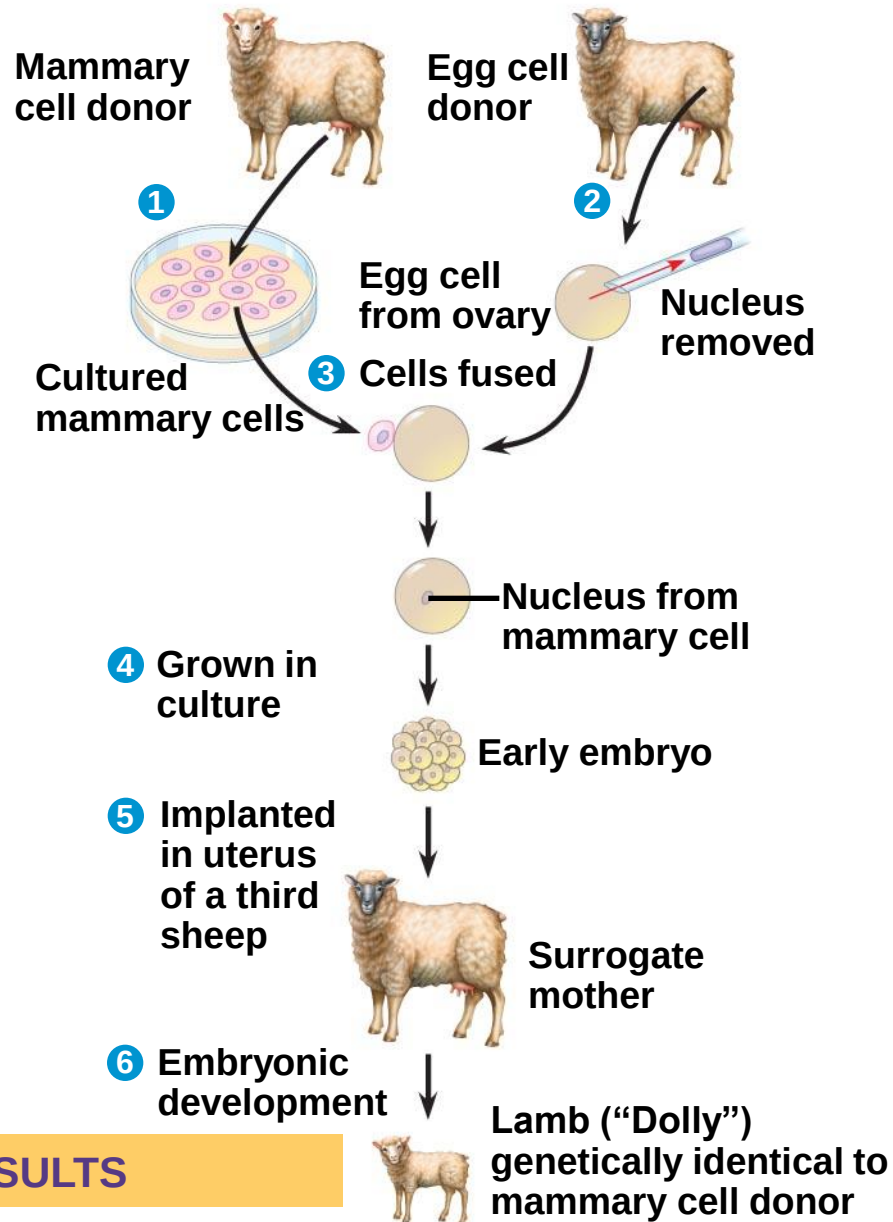


**Different culture
conditions**

**Different types of
differentiated cells**

Laura

TECHNIQUE



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- Since 1997, cloning has been demonstrated in many mammals, including mice, cats, cows, horses, mules, pigs, and dogs
 - CC (for Carbon Copy) was the first cat cloned; however, CC differed somewhat from her female “parent”
 - Identical human twins, naturally occurring “clones,” always differ somewhat.

Fig. 20-19



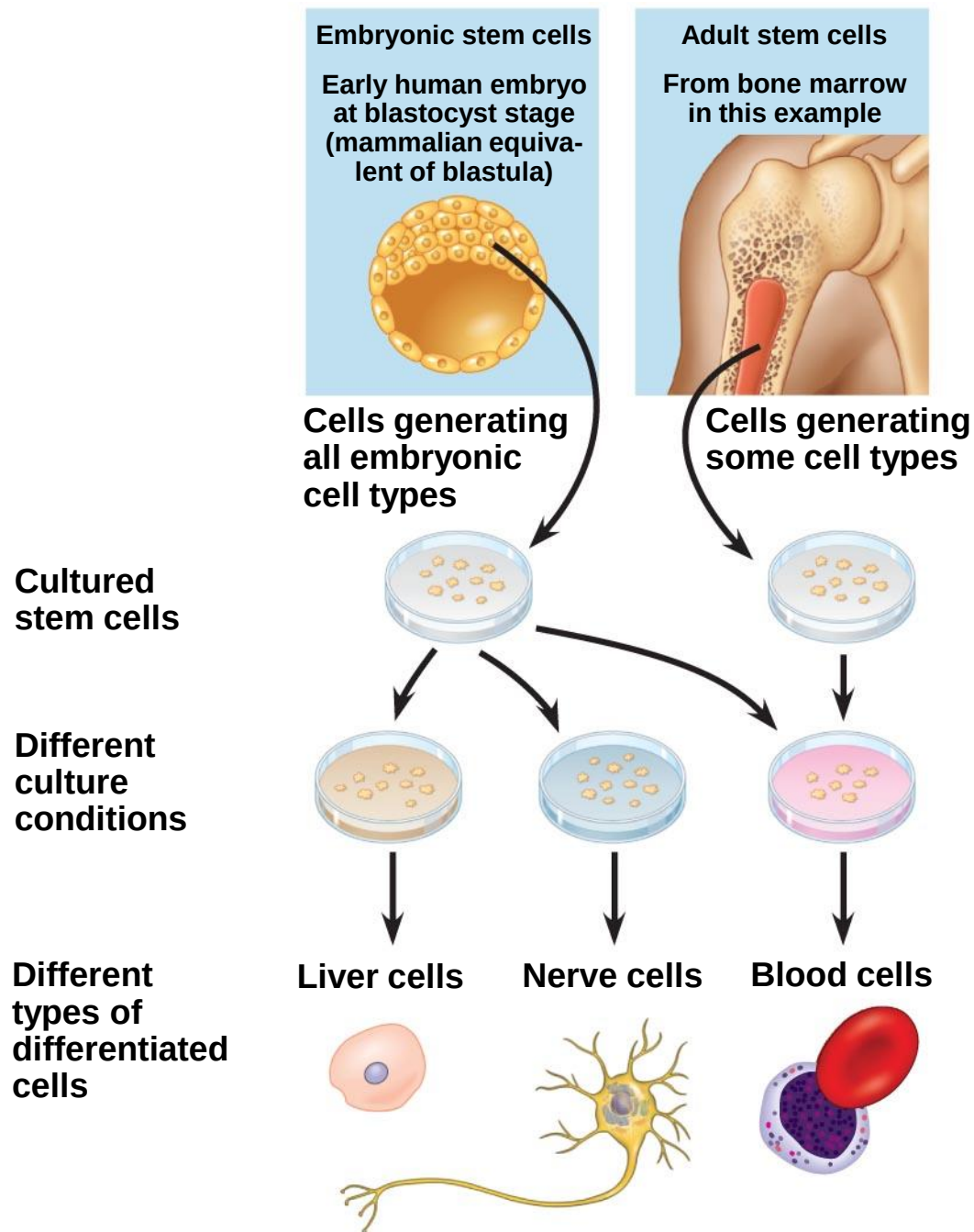
Problems Associated with Animal Cloning

- In most nuclear transplantation studies, only a small percentage of cloned embryos have developed normally to birth
- Many epigenetic changes, such as acetylation of histones or methylation of DNA, must be reversed in the nucleus from a donor animal in order for genes to be expressed or repressed appropriately for early stages of development

Stem Cells of Animals

- A **stem cell** is a relatively unspecialized cell that can reproduce itself indefinitely and differentiate into specialized cells of one or more types
- Stem cells isolated from early embryos at the blastocyst stage are called *embryonic stem cells*; these are able to differentiate into all cell types
- The adult body also has *adult stem cells*, which are able to give rise to many, but not all, cell types.

Fig. 20-20



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- The aim of stem cell research is to supply cells for the repair of damaged or diseased organs.
 - Although most people believe that the reproductive cloning of humans is unethical, opinions vary about the morality of *therapeutic cloning*, whose major aim is to produce embryonic stem cells to treat disease.

Concept 20.4: The practical applications of DNA technology affect our lives in many ways

- Modern biotechnology is making enormous contributions both to the diagnosis of diseases and in the development of pharmaceutical products.
- The identification of genes whose mutations are responsible for genetic diseases may lead to ways to diagnose, treat, or even prevent these conditions.

Medical Applications

- Susceptibility to many “nongenetic” diseases, from arthritis to AIDS, is influenced by a person’s genes.
 - Diseases of all sorts involve changes in gene expression within the affected genes and within the patient’s immune system.
 - Using DNA microarray assays and other techniques to compare gene expressions in healthy and diseased tissue can identify these changes and lead to the development of targets for prevention or therapy.

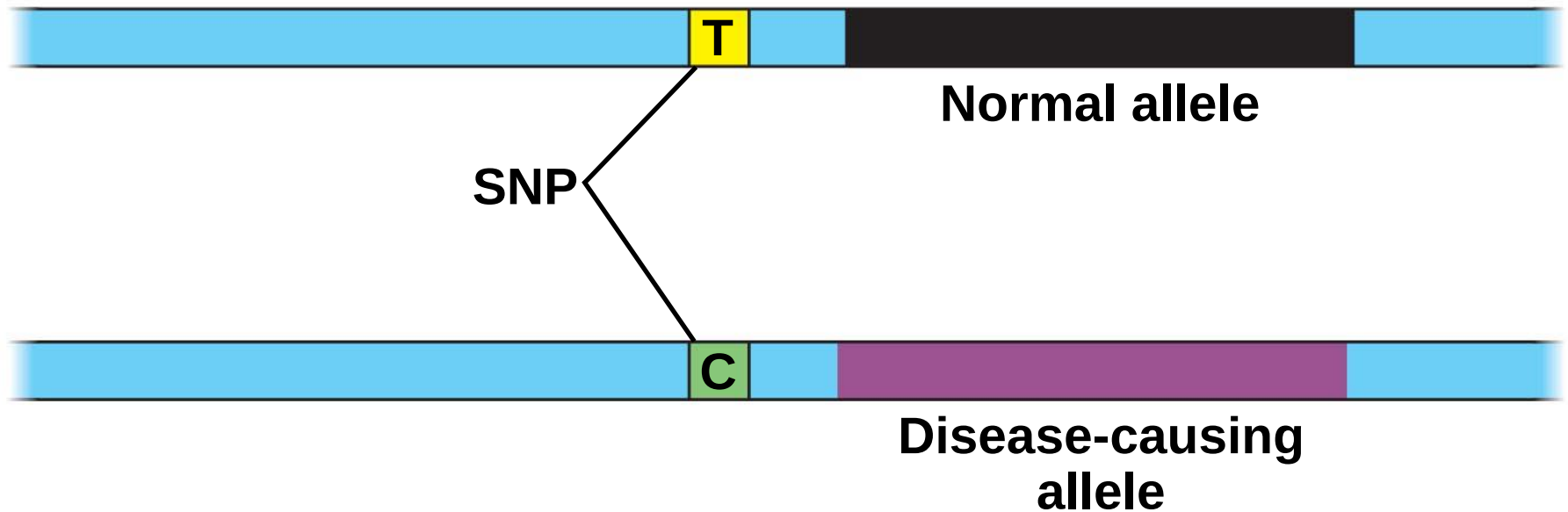
Diagnosis of Diseases

- Scientists can diagnose many human genetic disorders by using PCR and primers corresponding to cloned disease genes, then sequencing the amplified product to look for the disease-causing mutation
- Genetic disorders can also be tested for using genetic markers that are linked to the disease-causing allele

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- **Single nucleotide polymorphisms (SNPs)** are useful genetic markers
 - These are single base-pair sites that vary in a population
 - When a restriction enzyme is added, SNPs result in DNA fragments with different lengths, or **restriction fragment length polymorphism (RFLP)**

Single nucleotide polymorphisms (SNPs) as genetic markers for disease-causing alleles

DNA



Human Gene Therapy

- **Gene therapy** is the alteration of an afflicted individual's genes
- Gene therapy holds great potential for treating disorders traceable to a single defective gene
- Vectors are used for delivery of genes into specific types of cells, for example bone marrow
- Gene therapy raises ethical questions, such as whether human germ-line cells should be treated to correct the defect in future generations

Cloned gene

Retrovirus capsid

Bone marrow cell from patient

1 Insert RNA version of normal allele into retrovirus.

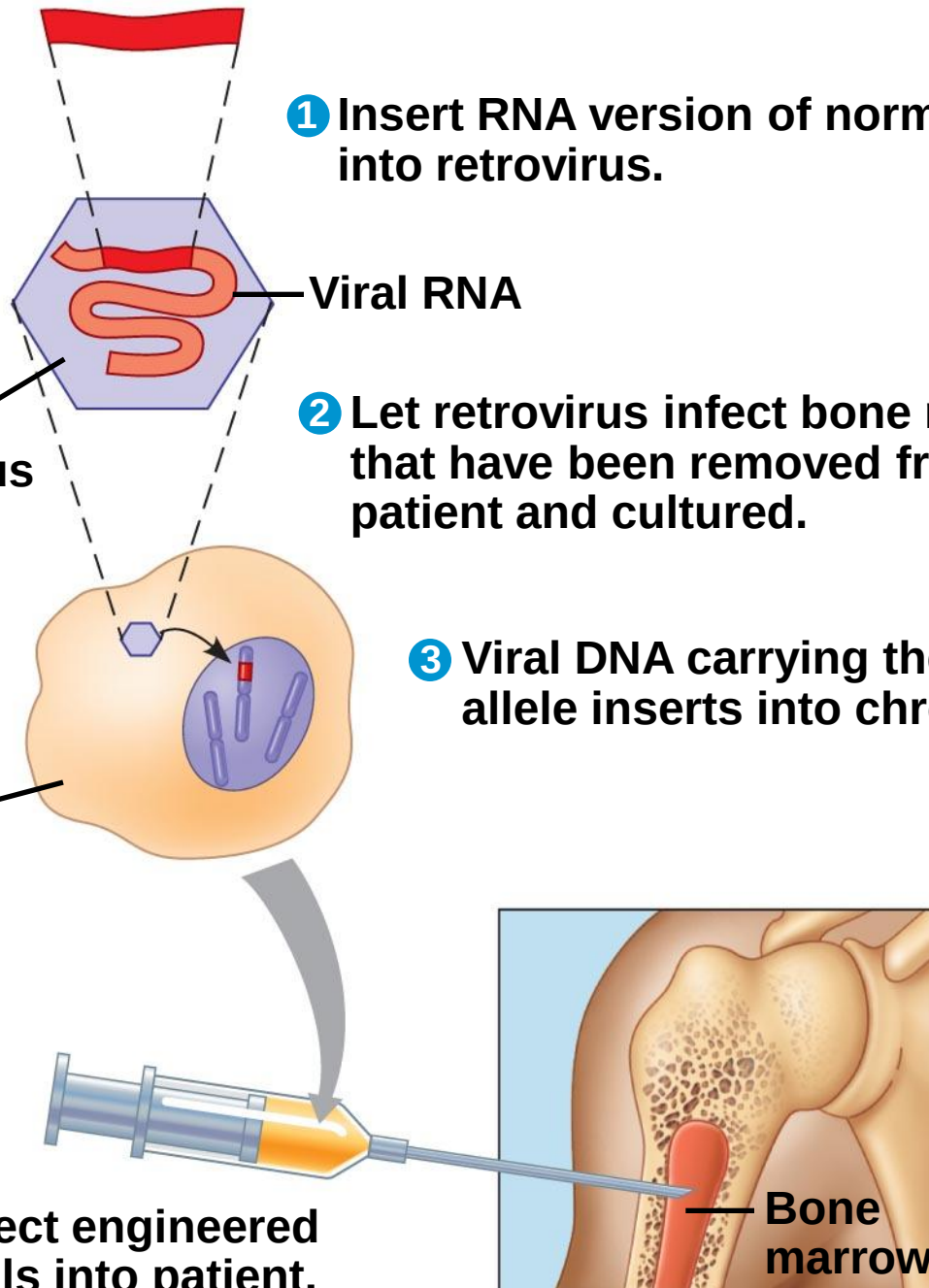
Viral RNA

2 Let retrovirus infect bone marrow cells that have been removed from the patient and cultured.

3 Viral DNA carrying the normal allele inserts into chromosome.

4 Inject engineered cells into patient.

Bone marrow



Pharmaceutical Products

- Advances in DNA technology and genetic research are important to the development of new drugs to treat diseases
- **Synthesis of Small Molecules for Use as Drugs**
 - The drug imatinib is a small molecule that inhibits overexpression of a specific leukemia-causing receptor
 - Pharmaceutical products that are proteins can be synthesized on a large scale

Protein Production in Cell Cultures

- Host cells in culture can be engineered to secrete a protein as it is made
- This is useful for the production of insulin, human growth hormones, and vaccines
- Cells in culture can also be used to produce vaccines, which stimulate the immune system to defend against specific pathogens.

Protein Production by “Pharm” Animals and Plants

- **Transgenic** animals are made by introducing genes from one species into the genome of another animal
- Transgenic animals are pharmaceutical “factories,” producers of large amounts of otherwise rare substances for medical use
- “Pharm” plants are also being developed to make human proteins for medical use

Fig. 20-23

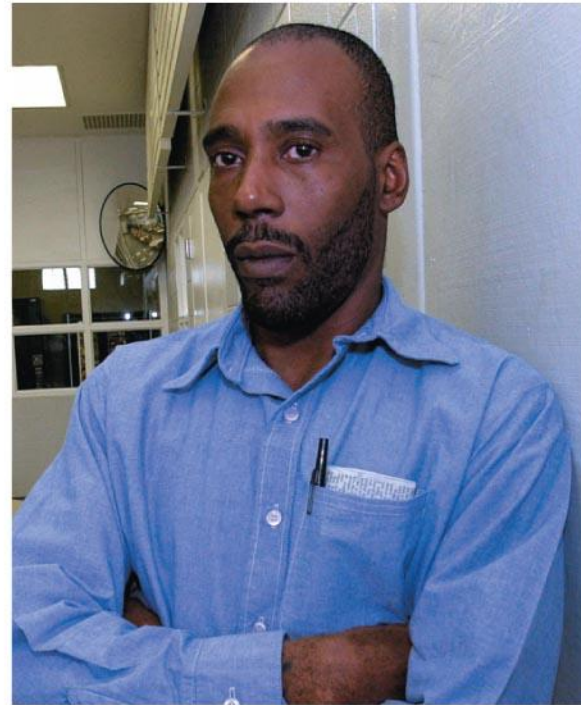


Forensic Evidence and Genetic Profiles

- An individual's unique DNA sequence, or **genetic profile**, can be obtained by analysis of tissue or body fluids
- Genetic profiles can be used to provide evidence in criminal and paternity cases and to identify human remains
- Genetic profiles can be analyzed using RFLP analysis by Southern blotting

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- Even more sensitive is the use of genetic markers called **short tandem repeats (STRs)**, which are variations in the number of repeats of specific DNA sequences
 - PCR and gel electrophoresis are used to amplify and then identify STRs of different lengths
 - The probability that two people who are not identical twins have the same STR markers is exceptionally small

(a) This photo shows Earl Washington just before his release in 2001, after 17 years in prison.



Source of sample	STR marker 1	STR marker 2	STR marker 3
Semen on victim	17, 19	13, 16	12, 12
Earl Washington	16, 18	14, 15	11, 12
Kenneth Tinsley	17, 19	13, 16	12, 12

(b) These and other STR data exonerated Washington and led Tinsley to plead guilty to the murder.

Environmental Cleanup

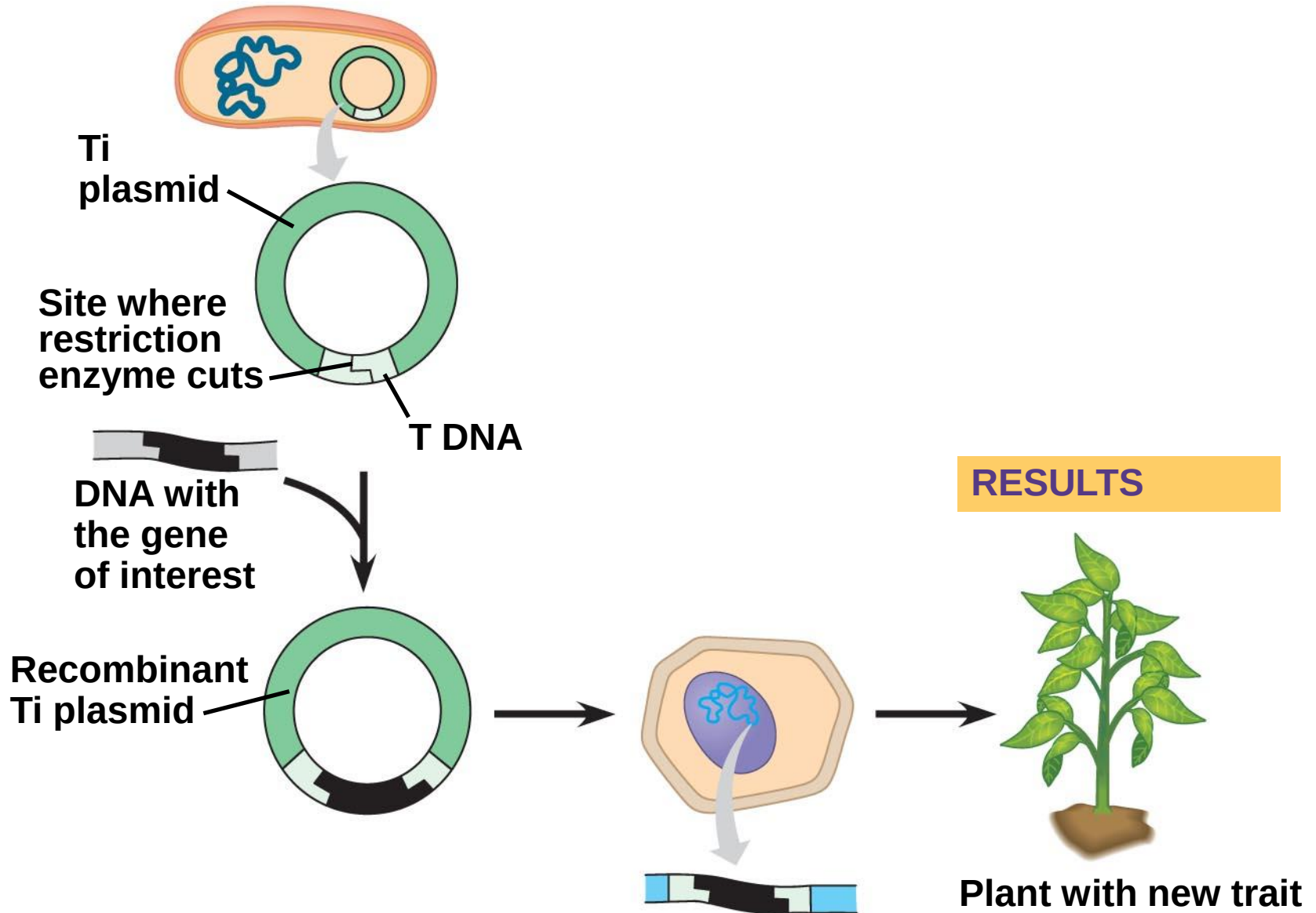
- Genetic engineering can be used to modify the metabolism of microorganisms
- Some modified microorganisms can be used to extract minerals from the environment or degrade potentially toxic waste materials
- Biofuels make use of crops such as corn, soybeans, and cassava to replace fossil fuels

Genetic Engineering in Plants

- Agricultural scientists have endowed a number of crop plants with genes for desirable traits
- The **Ti plasmid** is the most commonly used vector for introducing new genes into plant cells
- Genetic engineering in plants has been used to transfer many useful genes including those for herbicide resistance, increased resistance to pests, increased resistance to salinity, and improved nutritional value of crops

TECHNIQUE

Agrobacterium tumefaciens



Safety and Ethical Questions Raised by DNA Technology

- Potential benefits of genetic engineering must be weighed against potential hazards of creating harmful products or procedures
- Guidelines are in place in the United States and other countries to ensure safe practices for recombinant DNA technology

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- Most public concern about possible hazards centers on **genetically modified (GM) organisms** used as food
 - Some are concerned about the creation of “super weeds” from the transfer of genes from GM crops to their wild relatives

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- As biotechnology continues to change, so does its use in agriculture, industry, and medicine
 - National agencies and international organizations strive to set guidelines for safe and ethical practices in the use of biotechnology

You should now be able to:

1. Describe the natural function of restriction enzymes and explain how they are used in recombinant DNA technology
2. Outline the procedures for cloning a eukaryotic gene in a bacterial plasmid
3. Define and distinguish between genomic libraries using plasmids, phages, and cDNA
4. Describe the polymerase chain reaction (PCR) and explain the advantages and limitations of this procedure

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5. Explain how gel electrophoresis is used to analyze nucleic acids and to distinguish between two alleles of a gene
 6. Describe and distinguish between the Southern blotting procedure, Northern blotting procedure, and RT-PCR
 7. Distinguish between gene cloning, cell cloning, and organismal cloning
 8. Describe how nuclear transplantation was used to produce Dolly, the first cloned sheep

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9. Describe the application of DNA technology to the diagnosis of genetic disease, the development of gene therapy, vaccine production, and the development of pharmaceutical products
 10. Define a SNP and explain how it may produce a RFLP
 11. Explain how DNA technology is used in the forensic sciences

12. Discuss the safety and ethical questions related to recombinant DNA studies and the biotechnology industry

Detection of Mycoplasma contamination in mammalian cell culture

Discovered in 1898 and classified as

virus

**commonly called mycoplasma or PPLO
(pleuropneumonia like organisms), class of *Mollicutes* (soft skin)
with the families:**

***Mycoplasmataceae: Mycoplasma* (animal), *Ureaplasma* (animal)**

***Acholeplasmataceae: Acholeplasma* (animal, plant)**

***Spiroplasmataceae: Spiroplasma* (plant, rodent)**

***Anaeroplasma* (stomachs of ruminants)**

The majority of mycoplasma species contaminate human cell lines

***M. arginini* (bovine)**

***M. fermentans* (human)**

***M. hyorhina* (porcine)**

***M. orale* (human)**

***A. laidlawii* (bovine)**

The Effect of Mycoplasma

- Interference of cell growth rate
- Induction of morphological alteration (cyto pathology)
- Induction of chromosomal aberration chromosomal aberration
chromosomal aberration
- Influencing amino acid and nucleic acid metabolism
- Membrane alteration
- Transformation
- Associate to mammalian cell membranes

The Effect of Mycoplasma

- **Fast glucose reduction and formation of acids -> pH waste**
- **Arginine depletion -> inhibition of protein biosynthesis, cell division and growth**
- **Influence of immunological reactions (macrophage activation, inhibition of antigen presentation, induction of signal transduction)**
- **Influence of virus proliferation and the infection rate**
- **Decrease of the transfection rates by 5 % through electroporation**
- **Induction of leopard cells (condensation of the chromatins)**

Mycoplasma contamination through:

- ▶ **Cross-contamination from untested infected cells to other cell lines**
- ▶ **Primary cultures from the original tissue (incidence approximately 4 %) !! tissue graph**
- ▶ **Air borne microscopic aerosolization during pipetting**
- ▶ **Transfer of medium and/or cells during routine handling when more than one cell line is under the hood at a time**
- ▶ **The same bottle of medium is used for more than one cell line.**

Prevention:

- Good aseptic technique in conjunction with routine testing.
- Always try to work "**clean-to-dirty**" in order of handling cultures during a work day or week.
- Handle confirmed uncontaminated cells first,

Mycoplasma Diagnostic Methods

- DNA-binding Fluorescence Coloring Materials

 - Bisbenzimidazole, DAPI

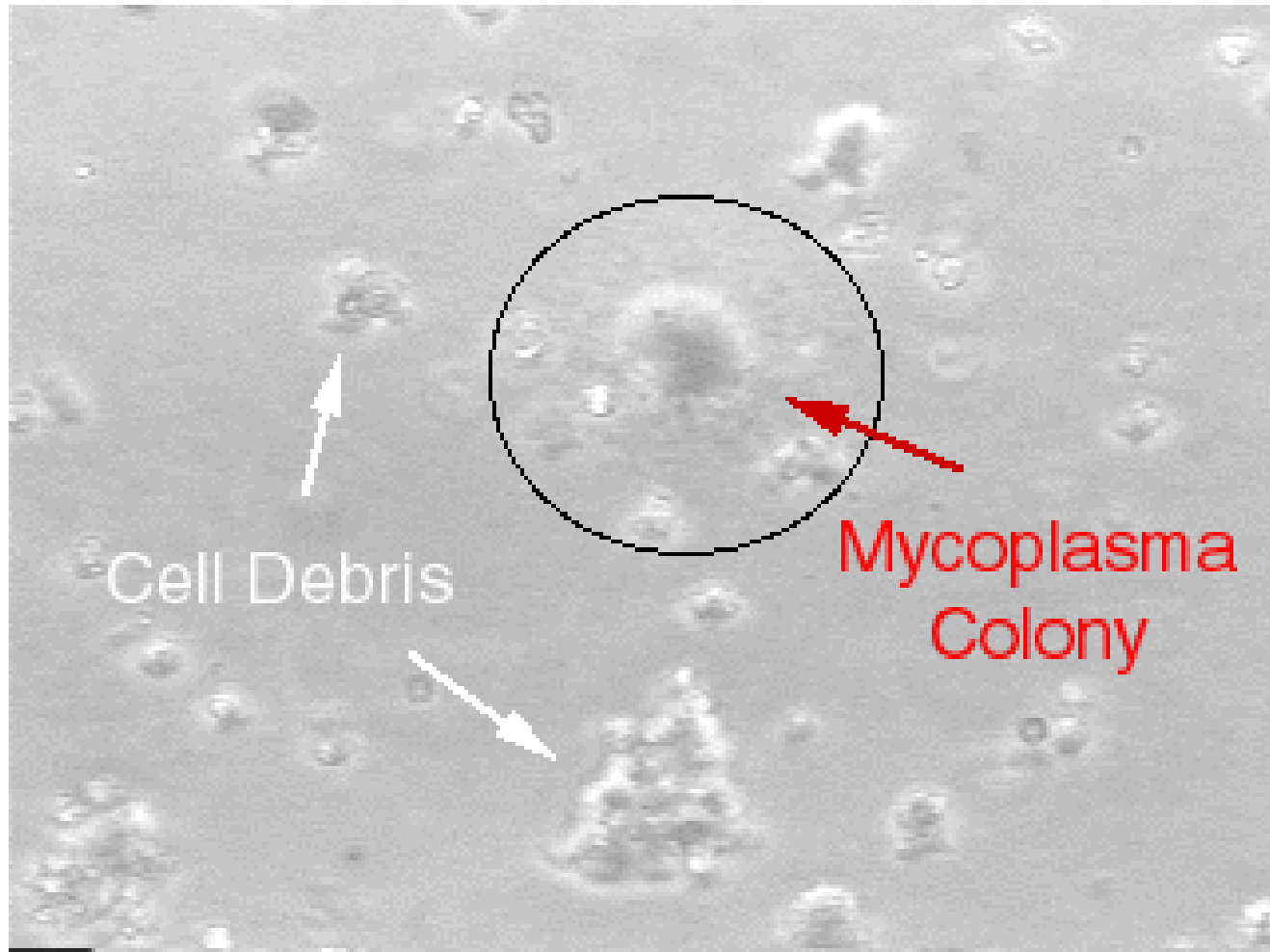
- Biochemical Verification Methods

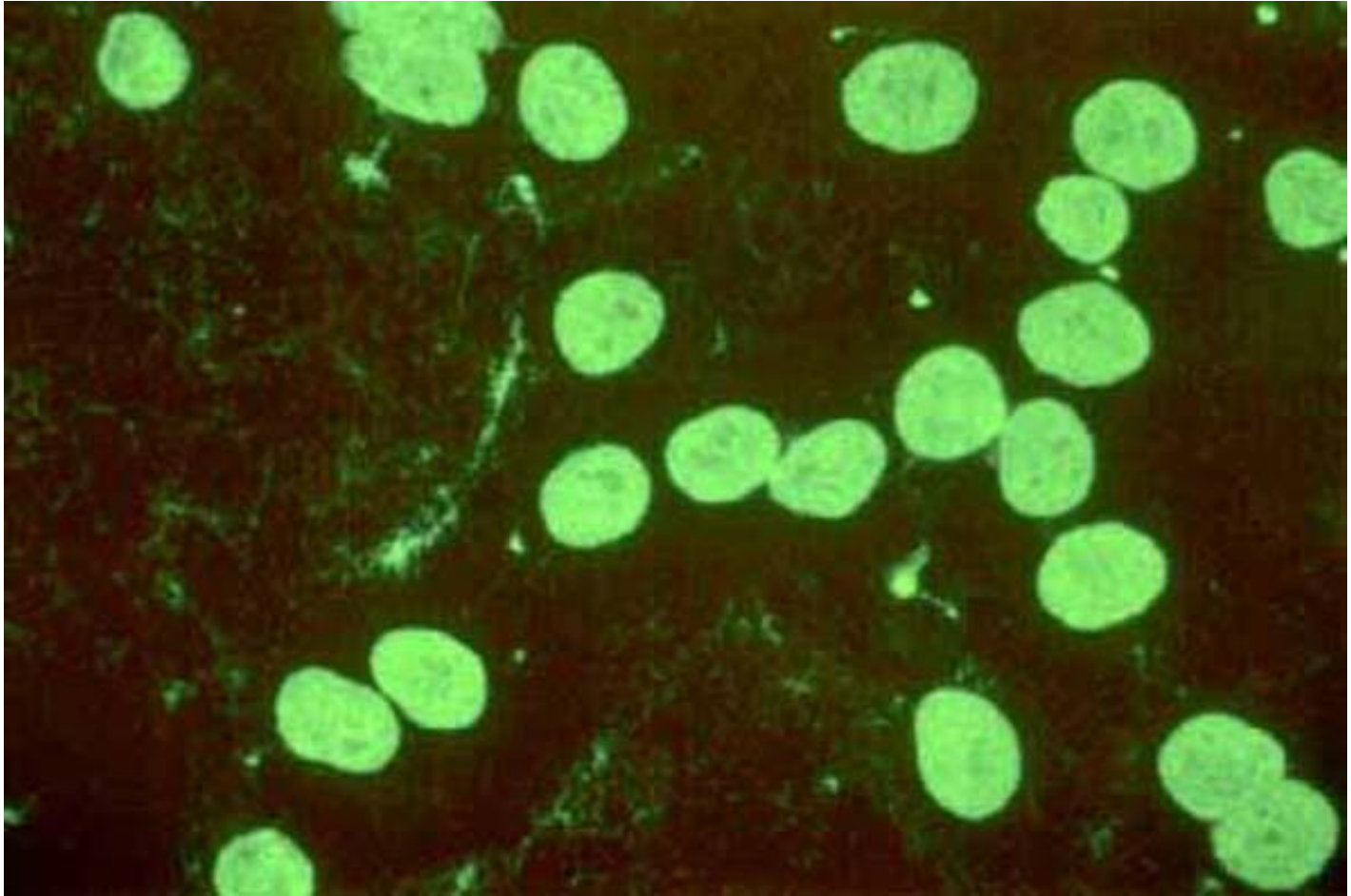
 - Adenosinophosphorylase test (6-MPDR-Test)

- Enzyme-Immuno Verification

- Cultivation Methods

- PCR-Technique





Mycoplasma Detection

5'- primers(Myco-5')

cgc ctg agt agt acg twc gc cgc ctg agt agt acg **tac**
gc

cgc ctg agt agt
acg **aac** gc

tgc ctg rtg agt aca ttc gc tgc ctg **atg** agt aca ttc
gc

tgc ctg **gtg** agt
aca ttc gc

cgc ctg agt agt atg ctc gc cgc ctg agt agt atg ctc
gc

Extraction of genomic DNA

1. Cells harvest from 6 mm dishor 25T flask Wash with PBS once
2. Transfer cells into a clean eppendorf
3. Centrifuge, 2000 rpm, 10 min
4. Discard PBS
5. Cell lyse with 300ul cell lysis buffer
6. Add 1.5ul RNAaseA sol , mix by invert 25x
7. Incubate 37°C, cool to room temperature

~~11. Take supernatant to a fresh tube~~

12. Add 300 ul of Isopropanol, mix by invert
50x till the
white thread appear

13. Centrifuge, 1 min

14. Wash with 1 ml 75% Ethanol

15. Centrifuge, 1 min, and air dry

16. DNA dissolve in 50 ul of 1X T.E buffer

17. Take 1 *ug* for PCR detection

Mycoplasma PCR test

primers	Myco-R1	6	ul	}	mixture1
	Myco-L1	3	ul		
dNTP (5mM)		2.5	ul		
10x Tag buffer		1.5	ul		
DNA		2	ul		
DDW		0	ul		

15 ul

95°C 7 min

Tag DNA polymerase 0.5 ul + 1 ul 10x buffer + 8.5 ul DDW **mixture2**
Pause →

65°C 2 min

72°C 5 min



95°C 5 sec

65°C 8 sec

72°C 16

1(each cycle)

32 cycles

10min



3 ul PCR product + ul 6X loading dye + 2ul TE



0.7% agarose gel electrophoresis

Mycoplasma DNA preparation

- Cells culture in antibiotic free medium for 2 weeks
- Collect 1 ml of supernatant
- Centrifuge 13000rpm, 5 min
- Resuspend pellet in 1 ml PBS
- Centrifuge again 13000rpm, 5 min
- Resuspend pellet in 100 ul PBS, heat 95°C, 15 min
- Add equal volume of phenol/chloroform, vortex
- Take upper layer
- Add 2.5 vol of 95% Ethanol, 1/10 vol 3M sodium acetate
- -20°C , over night
- Centrifuge 13000rpm, 10 min
- Wash pellet with 1 ml 75% ETOH
- Vacuum dry DNA and resuspend DNA in 50ul 1x TE

Mycoplasma PCR test

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72°C 5 min



95°C 5 sec

65°C 8 sec

72°C 17 sec

32 cycles

72°C 10min



5ul PCR product + 1ul 6X loading dye



1% agarose gel electrophoresis